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THE DETERIORATION OF THE BOVINE UDDER IN THE ABSENCE OF STREPTOCOCCI [1]

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INTRODUCTION

The rate of decline in the productive life of the modern dairy cow approximates 25 percent annually in the chief dairy regions of the United States and reaches 50 percent in large herds maintained by purchase. Many observers class deterioration of the udder as one of the important if not the most important factor in such "wastage" of dairy cows.

The role which certain of the streptococci play in the deterioration of the mammary gland of the cow has, during the past decade, been studied in each of the important dairy regions of the world. These bacteria are unanimously considered as the most important agency in the causation of udder troubles.

The significance of other bacteria, such as certain of the staphylococci, has recently been emphasized, and the literature of this phase of the question has been presented by Plastridge and his associates, as have the extended observations made by them (*11*). [2]

The possibility that there may be agents other than bacteria causing deterioration of the mammary gland has been suggested by Meigs and his associates (*7*) who studied the effect of milking machines in producing such changes in the milk as are indicative of chronic inflammation of the gland.

A filtratable virus was isolated from cows having mastitis by Broadhurst and her coworkers (*1*). Hastings and Beach (*3*, *4*) described cases of chronic mastitis in a herd of cows free from udder streptococci. The phrase "nonspecific mastitis" was used to indicate that no specific cause was found for the disturbances noted. The observations presented in these and in other papers indicate the complexity of the subject and emphasize the need of more extended observations made under a wide variety of conditions.

The present paper reports the observations made on a group of Holstein-Friesian heifers assembled previous to their first lactation period for the purpose of studying certain aspects of nutrition.

THE HERD AND THE METHODS

The 18 animals used in this experiment were kept in stable and yard constantly, and each received a ration which was not below that used on many dairy farms either from the quantitative or qualitative point of view. It thus seems that the abnormalities noted in the function and in the tissues of the udder cannot have been due to deficiencies in the feed.

[1] Received for publication November 20, 1939.

[2] Italic numbers in parentheses refer to Literature Cited, p. 155.

The cows were milked by a machine with care as to vacuum used and the length of time the machines were left on the cows. On 1 day of each week each cow was milked, morning and evening, by the machine so modified as to enable one to determine the yield of each separate quarter. The samples to be used for chemical determinations consisted of the first 30 cc. of milk from each quarter drawn into separate bottles, while those for bacteriological examination consisted of a few cubic centimeters drawn into a sterile container immediately after the milk for the chemical tests had been taken. The chlorine in the chlorides of the milk was determined by titration with silver nitrate, 10 cc. of milk diluted with 80 cc. of water in the presence of 2 cc. of a 10-percent solution of potassium dichromate being used. It is recognized that this method gives higher apparent values than that employed in previous studies (*3*, *4*). Since consideration of the data is confined to the group of animals observed, this limitation is of no significance.

The values given for catalase represent the volume of oxygen evolved from a mixture of 2 volumes of the sample and 1 volume of a 1-percent solution of hydrogen peroxide, expressed in percentage of the volume of milk used in the test. The method devised by the senior author for collecting and measuring the gas has been briefly described in a previous paper (*5*). Owing to the content of all body cells in catalase and to the relative, if not absolute, freedom of such fluids as the blood serum therefrom, the catalase values are believed to be an indirect measure of the content of the milk in intact body cells of all types and in cellular debris. The catalase value seems to have all the significance that an enumeration of cells has and is obtained with less effort.

CHLORINE AND LACTOSE

Davies (*2*) has shown that the chlorine content of the milk of different breeds is not the same, and it may be that the differences between individual animals of the same breed are significant. The observations of Davies show the fallacy of using any value such as 0.14 percent as an indication of disturbance in the udder when nothing is known about the normal level of chlorine in the milk of the animal in question. It seems safe to assume that the values for chlorine and catalase found in the milk produced by an animal in that part of the first lactation period immediately after the colostric stage, represent the base levels for that animal, and that persistent and marked deviations therefrom indicate a deterioration of the udder, and especially of individual quarters which show more marked deviations from the base level than other quarters, particularly when such changes are accompanied by a decreased quantity of milk. It is generally believed that the milk is isotonic with the blood, the freezing point of which is supposed to remain constant. The lactose and chlorides are believed to be the principal agents in governing the freezing point of milk, hence it follows that an increase in chlorine of 1 unit will necessitate a decrease in lactose of 11.1 units (*12*). A decrease in the percentage of lactose indicates a profound change in the procedure of milk synthesis in the udder.

In previous studies, in the absence of more definite reference points for chlorine and catalase values, use was made of arbitrary

values to separate the normal from the abnormal; thus 0.15 percent of chlorine was the upper normal limit for chlorine, and 50 the maximum value for normal catalase.

In the present studies more definite reference points have been available since the observations on the composition of the milk began at the beginning of the first lactation. It is assumed that the values found in the first 60-day interval after calving represent the base level of the animal in question, an assumption made more certain when the values of all quarters are much the same.

THE FINDINGS

BACTERIOLOGICAL FINDINGS

At the beginning of the study the bacteriological methods employed were those commonly used for the detection of streptococci. It soon became evident that this group of cows, like the one formerly studied, was free from udder streptococci, a condition which continued throughout the period of observation. Some students of chronic mastitis believe that streptococci are present in the mammary gland of every cow, and that failure to demonstrate them is owing to procedures used. It would seem that had streptococci been present in any animal of the group, they would have reached easily detectable numbers in some one of the animals at some time in the period of observation, if they were responsible for the abnormalities which appeared in the milk and tissues of certain quarters of some of the animals. Various procedures were followed in order to secure a more complete picture of the flora of the udders than could be obtained by the ordinary ones designed to detect streptococci.

The apparent lack of consistency in the occurence of any recognized type of bacteria has led to the tentative conclusion that no organism in the cultures was responsible for the changes noted in the secretion and tissues. The pathology of the abnormality, which in previous papers has been called nonspecific mastitis, has already been presented (*10*), as has its possible relationship to infection of the udder with streptococci (*9*).

DETAILED FINDINGS

Eleven of the original eighteen cows were observed during three lactation periods. During the first period observations were made each week. In the second and third periods the time between the samplings was somewhat longer on the average. It is impossible to present the detailed data for even a part of the group. In order that a sufficiently complete picture might be presented each lactation period has been divided into 60-day intervals and the average percentage of chlorine for all samples taken in any one interval has been calculated, as have the catalase value and daily production of milk by each quarter. The values for the last sample taken from each quarter in each period are also given in the tables. The use of averages eliminates the effect of transitory deviations from the normal and presents the trend of the continuous changes more clearly.

It is regretted that observations on the productivity of the quarters were not made in the first part of the first lactation period and the last part of the third. The data are, however, sufficiently complete to give a general picture of the conduct of each quarter. Of the

11 animals 4 were considered to be normal throughout the time of observation as regards the condition of their udders, while 7 became abnormal at some time and in some degree during the time of observation. The detailed records of 2 of the 4 normal cows and of 3 of the 7 abnormal cows are presented in the following tables.

Normal Cows

The record of cow 1 is given in table 1. It is to be noted that an increase in the percentage of chlorine is evident by the one hundred and eightieth day of the first lactation period; that the increase becomes greater as the period passes; that at the beginning of the second

TABLE 1.—*Yield, chlorine content, and catalase value of foremilk, at different stages in a lactation period and in different periods, for each quarter of normal cows 1 and 10*

COW 1

Lactation period and interval[1] No.	Quarter 1			Quarter 2			Quarter 3			Quarter 4		
	Yield per day	Chlorine	Catalase value	Yield per day	Chlorine	Catalase value	Yield per day	Chlorine	Catalase value	Yield per day	Chlorine	Catalase value
First:	*Lb.*	*Pct.*	*Pct.*	*Lb.*	*Pct.*	*Pct.*	*Lb.*	*Pct.*	*Pct.*	*Lb.*	*Pct.*	*Pct.*
1	------	0.110	9	------	0.105	8	------	0.107	10	------	0.113	10
2	------	.103	8	------	.105	16	------	.105	10	------	.097	12
3	4.3	.111	18	4.9	.114	18	4.8	.113	8	3.8	.111	7
4	3.6	.122	12	4.2	.133	13	4.2	.128	10	3.2	.132	11
5	2.8	.138	13	3.4	.137	10	3.5	.145	10	2.4	.146	13
6	2.2	.150	13	2.9	.142	10	3.0	.165	42	2.0	.123	22
361[2]	2.2	.177	32	3.2	.161	36	3.5	.216	150	2.5	.203	83
Second:												
1	5.5	.109	22	5.4	.124	23	6.1	.123	24	4.4	.123	28
2	4.0	.151	22	3.6	.159	21	4.7	.112	26	3.1	.119	28
3	2.6	.163	36	3.1	.137	22	3.6	.164	44	2.3	.114	27
4	1.7	.171	94	3.1	.173	50	2.3	.221	100	1.5	.176	56
5	.9	.224	56	1.8	.200	23	1.1	.252	93	1.2	.205	29
271[2]	.6	.247	68	1.4	.210	33	1.4	.254	94	1.1	.220	31
Third:												
1	8.7	.136	44	10.2	.142	41	7.5	.168	71	7.3	.115	60
2	7.3	.160	58	9.0	.150	50	7.1	.159	47	6.4	.151	40
3	3.6	.219	110	5.3	.183	60	5.1	.198	70	4.1	.207	65
4	------	.268	159	------	.229	93	------	.236	115	------	.254	132
204[2]	------	.255	167	------	.229	87	------	.233	119	------	.259	163

COW 10

First:												
1	------	0.115	5	------	0.115	4	------	0.112	5	------	0.113	5
2	7.6	.123	9	8.7	.121	4	9.3	.116	5	7.6	.121	8
3	6.4	.123	6	7.4	.132	19	7.8	.129	15	6.3	.127	6
4	6.1	.150	16	7.2	.161	31	7.1	.154	17	6.3	.151	10
5	5.4	.144	12	6.1	.151	26	6.6	.142	8	5.1	.146	8
6	3.4	.186	69	4.6	.168	45	4.3	.168	22	3.2	.180	31
359[2]	2.6	.234	80	3.2	.212	104	2.5	.207	39	1.7	.226	36
Second:												
1	9.6	.110	18	10.2	.127	41	11.2	.111	19	8.0	.108	13
2	8.5	.131	28	8.1	.134	32	10.7	.118	8	9.1	.156	30
3	7.8	.144	50	7.3	.150	52	9.6	.134	23	7.5	.131	33
4	6.6	.165	48	6.2	.170	44	8.0	.164	33	6.7	.150	21
5	4.2	.200	38	4.3	.204	36	4.3	.190	34	4.3	.175	81
6	3.8	.227	53	2.5	.182	58	3.9	.230	42	2.8	.214	39
330[2]	4.5	.259	94	2.7	.280	104	3.6	.287	84	2.8	.245	47
Third:												
1	9.6	.166	37	11.7	.157	20	13.6	.153	15	9.2	.152	21
2	9.5	.188	92	12.3	.163	25	13.7	.146	12	8.7	.147	18
3	------	.180	60	------	.160	24	------	.156	17	------	.163	35
4	------	.187	82	------	.168	44	------	.168	29	------	.158	17
5	------	.197	41	------	.199	33	------	.192	32	------	.171	20
6	------	.202	50	------	.196	38	------	.208	42	------	.187	24
309[2]	------	.207	47	------	.202	33	------	.205	31	------	.185	19

[1] Interval is 60 days.
[2] The day of last sampling. It also gives length of the last interval, e. g., in second period for cow 1, 4 intervals plus 31 equals 271 days.

period it is higher in three quarters than at the beginning of the first; and that the increase is earlier in the second period than in the first and reaches a higher level at the end. The percentage of chlorine is higher at the beginning of the third period than at the first part of the second. A parallel increase during any period, and from period to period, occurs in the case of catalase.

The data in table 2 are the averages of all quarters for chlorine and catalase for the first 60-day interval of each period, and for the fourth interval. The samples from which the data were obtained consisted of the first 30 cc. of milk from each quarter. In previous work both the foremilk and the entire quantity produced by a quarter were examined. The entire milk of a quarter tended to show the same progressive change in chlorine and catalase content as did the foremilk, but to a less extent. This indicates that the change in composition is not caused by increased excretion of fluid from the membranes lining the milk ducts, but is the result of some modification in the synthesis of the milk.

TABLE 2.—*Chlorine content and catalase value of foremilk during 2 intervals of 3 lactation periods for normal cows 1 and 10*

Lactation period	Cow 1				Cow 10			
	Interval 1		Interval 4		Interval 1		Interval 5	
	Chlorine	Catalase value	Chlorine	Catalase value	Chlorine	Catalase value	Chlorine	Catalase Value
	Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
1	0.109	9	0.129	12	0.114	5	0.146	13
2	.120	24	.185	75	.114	23	.192	22
3	.140	54	.247	125	.157	47	.190	31

It is evident from the values given that cow 1 would have been judged as definitely mastitic during the last of the second period, and during the third after the one hundred and twentieth day, since a composite sample would have given chlorine and catalase values in excess of commonly accepted standards. It is believed that cow 1 may be considered normal, on the basis that all quarters change in much the same manner throughout each period and from period to period.

Little (*6*) presented data of similar significance. His observations were made on cows yielding milk which failed to show mastitis streptococci, or alteration due to staphylococci or miscellaneous organisms. The data were for groups of cows and showed an increase in chlorine and in leucocytes as the lactation period passed, and from one period to the succeeding. It was also noted by Little that the bacterial content increased in a period, and from period to period, a conclusion which the findings of the present writers tend to confirm. It thus seems that in some cows with apparently normal udders there is a slowly progressive change which is reflected in the composition of the milk, and in a lessened inhibitory power toward bacteria. It may be that herein lies the explanation for the higher incidence of infection of the udder with streptococci in older than in younger animals, rather than in greater opportunity for infection because of longer time. Cow 1 produced 5,193 pounds of milk in 270 days of the

first period and only 3,306 in the same interval of the second. During 205 days of the third period, 5,093 pounds were produced as compared with 2,961 in the same number of days of the second. The cause of the small production in the second period is unknown, and was not evident in the appearance of the animal.

The record of cow 10 is also presented in tables 1 and 2. The changes are similar to those noted in cow 1, but are of less magnitude in any interval. The udder of this animal resisted change to a greater extent than did that of cow 1. This resistance is reflected in the yield of milk, which increased more than 22 percent from the first to the third period. The two remaining normal animals showed changes similar to the two cows just discussed. The quantitative shifts in composition were greater than those in the milk of cow 10 and less than those in that of cow 1. It seems unnecessary to present the detailed data.

Abnormal and Intermediate Animals

The record of cow 18 is given in table 3. The outstanding points of the record for the first period are the high chlorine and catalase after the three hundredth day, and the apparent normal values previous to this time. The extent of changes was much the same in each quarter. In the first interval of the second period only one quarter, 4, is normal, the other three having an average chlorine content of 0.209 percent as compared with 0.127 percent in the first period. The abnormality increased in intensity during the third period in quarters 1 and 2; quarter 3 changed from abnormal to normal; and quarter 4 from normal to abnormal in the third period. This is one of the few cases of reversion to normal noted in the writers' observation of two groups of cows.

Quarter 3 which changed from abnormal to normal, produced in the second interval of the second period 24.6 percent of the total yield of milk, and in the third period, 33.9 percent. Quarter 4, normal in the second period, and abnormal in the third, produced 25.5 and 21.2 percent in the second interval of these periods, respectively, the changes in the milk were not the same in the different quarters, a condition which may have indicated the presence of some agent not present in cows 1 and 10. The total production for the first 250 days of each period was 7,034, 7,342, and 6,389 pounds, respectively.

The record of cow 8 is also given in table 3. The quarters do not present the same picture in the first period; quarter 3 was abnormal after the first interval, and quarter 1 after the second. The base level of chlorine for the animal was 0.119 percent. In the first interval of the second period, 0.260 percent of chlorine was found, and 0.278 percent in the third period. The values for catalase were 24, 137, and 126. The cow produced in the first 90 days of each of the three periods 4,437, 3,986, and 4,524 pounds of milk, respectively.

The record of cow 7, given in table 3, represents an intermediate between the normal and the distinctly abnormal. The third quarter in the third period produced milk far exceeding the usual standards of chlorine and catalase. The yield of milk decreased from 7,006 pounds in the first 250 days of the first period to 6,584 pounds in the second, and to 5,451 pounds in the third, evidencing a progressive deterioration of the udder which is reflected in the composition of the milk in regard to chlorine and catalase.

TABLE 3.—*Yield, chlorine content, and catalase value of foremilk, at different stages in a lactation period and in different periods, for each quarter of abnormal cows 18 and 8, and intermediate cow 7*

ABNORMAL COW 18

Lactation period and interval[1] No.	Quarter 1			Quarter 2			Quarter 3			Quarter 4		
	Yield per day	Chlorine	Catalase value	Yield per day	Chlorine	Catalase value	Yield per day	Chlorine	Catalase value	Yield per day	Chlorine	Catalase value
	Lb.	*Pct.*	*Pct.*	*Lb.*	*Pct.*	*Pct.*	*Lb.*	*Pct.*	*Pct.*	*Lb.*	*Pct.*	*Pct.*
First:												
1	------	0.132	11	------	0.122	11	------	0.127	6	------	0.131	11
2	7.8	.129	9	9.8	.123	6	6.9	.137	9	5.8	.137	8
3	6.5	.145	12	7.2	.129	3	6.4	.141	9	5.5	.142	11
4	6.8	.157	7	7.6	.177	24	5.9	.165	10	5.1	.169	15
5	3.9	.174	21	5.3	.216	64	4.2	.187	27	3.5	.172	15
6	1.1	.361	116	1.6	.339	123	1.2	.343	135	1.1	.313	123
319[2]	.5	.378	98	.9	.360	110	------	.376	133	------	.329	138
Second:												
1	9.9	.200	98	11.2	.202	108	10.4	.225	116	10.2	.125	27
2	7.3	.207	140	9.1	.229	115	8.1	.249	123	8.4	.126	21
3	5.8	.207	123	7.5	.217	131	6.3	.233	129	7.0	.136	18
4	5.0	.240	108	6.5	.242	92	5.0	.257	74	6.4	.157	18
5	------	.366	190	------	.370	176	------	.364	190	------	.287	122
257[2]	------	.398	180	------	.396	192	------	.400	188	------	.371	192
Third:												
1	9.5	.271	158	7.2	.317	165	12.6	.177	38	7.7	.250	150
2	6.6	.271	173	6.1	.340	208	9.6	.184	54	6.0	.280	151
3	------	.242	129	------	.275	146	------	.175	33	------	.272	158
4	------	.270	126	------	.240	94	------	.196	62	------	.281	106
5	------	.276	133	------	.260	100	------	.307	127	------	.315	136
283[2]	------	.287	139	------	.275	116	------	.310	139	------	.283	119

ABNORMAL COW 8

First:												
1	6.2	0.121	9	9.2	0.129	16	8.7	0.140	51	7.0	0.127	20
2	5.4	.159	13	7.5	.138	6	6.2	.232	63	5.7	.170	26
3	3.3	.206	31	6.2	.145	10	4.6	.279	107	4.6	.185	29
4	2.1	.264	104	4.1	.222	44	3.7	.258	113	3.2	.204	64
5	1.0	.244	108	2.5	.247	94	1.3	.260	117	1.5	.225	107
268[2]	1.0	.253	102	1.9	.257	97	.7	.265	126	1.5	.265	118
Second:												
1	6.8	.263	88	8.9	.289	182	9.1	.272	156	7.5	.216	121
2	5.4	.341	137	7.4	.287	133	7.6	.320	135	6.4	.303	131
3	1.8	.362	188	3.7	.320	182	4.1	.333	182	3.1	.330	190
4	------	.329	143	------	.309	137	------	.310	113	------	.361	185
194[2]	------	.317	172	------	.320	139	------	.315	144	------	.345	191
Third:												
1	------	.256	100	------	.321	163	------	.290	150	------	.246	91
2	------	.285	152	------	.330	187	------	.287	139	------	.176	102
3	------	.300	132	------	.297	143	------	.264	88	------	.261	89
4	------	.299	133	------	.196	120	------	.293	124	------	.297	126
204[2]	------	.287	141	------	.282	131	------	.276	135	------	.286	119

INTERMEDIATE COW 7

First:												
1	------	0.118	15	------	0.120	10	------	0.127	22	------	0.118	32
2	7.6	.110	7	8.3	.117	10	7.8	.130	14	7.4	.107	6
3	6.8	.114	7	7.4	.129	15	6.8	.148	23	6.5	.117	8
4	5.2	.140	9	6.0	.152	17	5.5	.183	30	5.3	.135	10
5	2.8	.152	23	3.0	.169	23	3.0	.176	28	3.0	.165	42
283[2]	.9	.200	60	1.3	.214	37	1.2	.212	38	1.0	.209	51
Second:												
1	9.4	.081	11	10.2	.093	19	8.5	.104	30	8.0	.084	10
2	8.0	.124	91	9.2	.104	44	7.6	.111	27	7.5	.102	24
3	4.8	.188	140	7.3	.118	38	6.4	.151	55	6.4	.140	17
4	3.2	.244	144	5.8	.163	46	5.6	.208	73	6.2	.158	33
5	.9	.286	------	2.1	.317	------	2.1	.302	------	1.2	.279	------
250[2]	.9	.286	------	2.1	.317	------	2.1	.302	------	1.2	.279	------
Third:												
1	8.4	.143	32	9.9	.170	34	7.3	.202	89	5.1	.175	91
2	7.2	.138	29	7.3	.170	28	5.5	.203	72	4.7	.170	30
3	------	.146	53	------	.209	64	------	.251	125	------	.189	53
4	------	.211	93	------	.237	105	------	.244	146	------	.240	84
5	------	.276	136	------	.287	126	------	.304	188	------	.290	102
243[2]	------	.276	136	------	.287	126	------	.304	188	------	.290	102

[1] Interval is 60 days.

[2] See footnote 2, table 1.

Increases in Chlorine

Table 4 shows that the milk produced by the individual quarters of each animal has, in general, a higher content of chlorine in the third than in the first interval of any period. This is the increase usually ascribed to late lactation. The data show that evidences of such changes appear earlier than is commonly supposed.

Table 4.—*Chlorine content of foremilk from each quarter of five cows at different intervals of the same lactation period and in different periods*

Cow No.	Period	First interval				Third interval			
		Quarter 1	Quarter 2	Quarter 3	Quarter 4	Quarter 1	Quarter 2	Quarter 3	Quarter 4
		Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
1	1	0.110	0.105	0.107	0.113	0.111	0.114	0.113	0.111
	2	.109	.124	.123	.123	.163	.137	.164	.114
	3	.136	.142	.168	.155	.219	.183	.198	.207
10	1	.115	.115	.112	.113	.123	.132	.129	.127
	2	.110	.127	.111	.108	.144	.150	.134	.131
	3	.166	.157	.153	.152	.180	.160	.156	.163
7	1	.118	.120	.127	.118	.114	.129	.148	.117
	2	.081	.093	.104	.084	.188	.118	.151	.140
	3	.143	.170	.202	.175	.146	.209	.251	.189
8	1	.121	.129	.140	.127	.206	.145	.279	.185
	2	.263	.289	.272	.216	.362	.320	.333	.330
	3	.256	.321	.290	.246	.300	.297	.264	.261
18	1	.132	.122	.127	.131	.145	.129	.141	.142
	2	.200	.202	.225	.125	.207	.217	.233	.136
	3	.271	.317	.177	.250	.242	.275	.175	.272

A comparison of the data shows the increase from period to period even in the animals classed as normal in all their quarters. In the case of the second period as related to the first the increase is slight or absent, but generally marked in the third period as compared with the first and second.

Table 5 shows that increases in catalase similar to those in chlorine occur in the milk of individual quarters in any period and from one period to the next. The increase in the cellular content of milk as the lactation period passes was noted by Russell and Hoffman (*13*) and more recently by Little. Russell and Hoffman also noted the increase in the cell content of the milk as the animal became older. According to prevailing theories chlorine and lactose must change together in order to maintain the milk in osmotic balance with the blood. The question as to which is the cause and which the effect cannot be answered. No matter what the answer, the fundamental process of lactose manufacture is interfered with. It is easy to conceive that the decrease in lactose is the cause of the increase in chlorine in such animals as the writers have considered normal, while in the case of a definite mastitis caused by some foreign element present in only a part of the quarters, the influx of chlorine may cause a disturbance in the physiology of lactose manufacture. Two phenomena are illustrated in the animals discussed herein: (1) An inflammatory process; (2) a physiological one, a part of the reproductive cycle of the animal. The latter seems to be accentuated from period to period. The milk becomes lower in nutritive value, and hence from certain points of view the change may be looked upon as one of deterioration of the udder.

TABLE 5.—*Catalase value of foremilk from each quarter of five cows at different intervals of the same lactation period and in different periods*

Cow No.	Period	First interval				Third interval			
		Quarter 1	Quarter 2	Quarter 3	Quarter 4	Quarter 1	Quarter 2	Quarter 3	Quarter 4
		Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
1	1	9	8	10	10	18	18	8	7
	2	22	23	24	28	36	22	44	27
	3	44	41	71	60	110	60	70	65
10	1	5	4	5	5	6	19	15	6
	2	18	41	19	13	50	52	23	33
	3	37	20	15	21	60	24	17	35
7	1	15	10	22	32	7	15	23	8
	2	11	19	30	10	140	38	55	7
	3	32	34	89	91	53	64	125	53
8	1	9	16	51	20	31	10	107	29
	2	88	182	156	121	188	182	182	190
	3	100	163	150	91	132	143	88	89
18	1	11	11	6	11	12	3	9	11
	2	98	108	116	27	123	131	129	18
	3	158	165	38	150	129	146	33	158

The histological changes found in the tissues of such quarters as 1 and 2 of cow 18 in the second and third periods have been described in a previous paper (*10*).

MILK PRODUCTION

The influence of any disturbance in the udder on milk production is difficult to determine since so many factors may be concerned. It has been stated earlier that an abnormal quarter seems to produce less than the share of milk it produced when normal. It is possible to calculate from the data presented the part of the total milk produced by each quarter at any interval in any period. The question of compensation cannot be answered in any case, and thus the question of total production in its relation to the condition of the udder, as indicated by changes in the milk, may be the most significant fact. Eleven cows were observed during three periods in such detail as to form a basis for judgment as the condition of the udders. The 11 animals were placed in 3 groups: One containing the normal cows, another the most abnormal, and a third of intermediate condition. The production in the same number of days in the first and second periods is presented in table 6 in percentages of increase or decrease and a similar comparison is made for the second and third periods.

TABLE 6.—*Percentage changes in the production of milk in successive lactation periods by cows classified as normal, slightly abnormal, and very abnormal*

Classification	Number	Increase (+) or decrease (−) in production from—	
		First to second period	Second to third period
		Percent	*Percent*
Normal cows	4	+16	+24
Slightly abnormal cows	5	+4	+12
Very abnormal cows	2	−3	+2

DISCUSSION

The tissue of the lactating udder of the cow seems very responsive to stimuli, or, in other words, is easily irritated. It is conceivable that a mild irritant acting through long periods of time may cause a noticeable disturbance in function and in structure. The milking machine under certain conditions and on certain cows may exert such a continuously irritating effect. Bacteria of certain types may produce slightly injurious compounds which, acting over considerable periods, may cause a detectable effect, and especially when they grow more freely than in the usual animal. The writers' observations suggest that the bacteria are held in check more completely during the first lactation period than during subsequent ones.

Many agents may be cooperating to produce such changes as are described in this paper, and it may well be that students of mastitis are thinking in too narrow terms, a danger presented by Shope (*14, p. 932*):

> Another fact of considerable interest that was not emphasized is that in the case of each one of the seven diseases discussed a simple "one agent" causation was in the beginning considered probable or proved. Some minor discrepancy or fortuitous observation led eventually to the discovery that two agents were involved. This seems to indicate that those who are studying infectious diseases are thinking largely in terms of one instead of two causal agents. They are still under the influence of the spirit of Koch's postulates and find it difficult to abandon even occasionally the concept that for each infectious disease there must be a single specific etiologic agent. If there is anything at all to be learned from present knowledge of the complex infections, it is that an infectious agent must fully explain and account for all of the features of a disease with which it is associated before it is accepted as the sole cause of that disease. Investigators must think more often in terms of two factors if they are to gain full understanding of all the infectious diseases.

The observations presented in this and in previous papers have raised questions rather than answered them. It is evident that dairy cows can be managed intelligently, as regards the avoidance of deterioration of the udder, only with more complete knowledge of the factors involved and of their relations to each other. Munch-Petersen (*8, p. 238*) concludes his recent survey of the literature on bovine mastitis with the statement that much of the work on the subject has been abortive because of lack of fundamental knowledge regarding many aspects of the subject. He emphasizes the necessity of additional research work on a number of points and states that among the conditions which must obtain in order to secure the necessary data—

> the herd must be newly established, and be founded by heifers coming to their first lactation, and must be handled for several seasons for the convenience of the research workers and the acquisition of knowledge relating to mastitis, rather than as a commercial concern; and the research workers must have laboratory facilities actually on the premises.

The observations made at this station on two groups of cows emphasize the need which Munch-Petersen presents.

SUMMARY

Eleven Holstein-Fresian cows were observed during the first, second, and third lactation periods as to the chemical composition of the foremilk and the number and kinds of bacteria therein.

This group was not in contact with other cattle during the period of study. The ration of any member of the group was uniform throughout the period and was the equal, quantitatively and qualitatively, to the rations used on many dairy farms. The cows were milked by a machine, operated by an experienced operator. On 1 day of each week each cow was milked with the machine so arranged as to permit a determination of the yield of each quarter. Samples were taken at weekly intervals throughout most of the period of observation and were examined as to their chlorine content and catalase value and for number and kinds of bacteria. It is considered that the values found in that part of the first lactation period immediately after the colostric stage represent the normal or base level of chlorine and catalase for the individual. On account of the relation existing between chlorides and lactose, the two substances that keep the milk serum in isotonic balance with the blood serum, a marked and persistent increase in the chlorine content of the milk implies some degree of deterioration of the udder, as does an increase in catalase, which is considered to be an indirect measure of the content of the milk in cells or cellular debris.

In the case of each cow the percentage of chlorine and catalase increased as each lactation period passed, and from period to period.

In some cases the increase became evident at about the same time in each quarter and was of much the same magnitude in each. The chlorine content and catalase value then tend to be much the same during the early part of the second period as during the first part of the first lactation period, but reach significantly higher levels during the early part of the third period. The average percentages of chlorine and catalase noted in the middle of the third lactation period were above the values usually considered as normal. The yield of milk showed from period to period the expected or normal increase.

In other cases the normal level of chlorine and catalase of the early part of the first lactation period was exceeded in the early part of the second as well as in the first part of the third period. In such animals the milk produced by the different quarters of the udder did not change at the same time and to the same extent. The deterioration of the udders was then more marked than in the case of the animals classed as normal, as was shown by the fact that the yield of milk did not show the expected increase from the first to the second and from the second to the third periods.

The results of the bacteriological studies, continued throughout the three lactation periods and obtained by various techniques, supplied no explanation for the changes noted. The udder of each animal remained free from streptococci through the three lactation periods. No other organism was found with sufficient consistency and in sufficient number to be apparently responsible for the changes noted in the milk and in the structure of the udder. The observations indicate that the causes of mastitis or of deterioration of the udder may be more complex than is now supposed and indicate the need of a more detailed and more prolonged study of the subject.

LITERATURE CITED

(1) Broadhurst, Jean, Cameron, Gladys, and MacLean, M. Estelle. 1939. A filtratable virus isolated from mastitis cows. Cornell Vet. 29: 261–270, illus.

(2) DAVIES, W. L.
1938. THE CHLORIDE CONTENT OF MILK. Jour. Dairy Res. 9: [327]–335, illus.
(3) HASTINGS, E. G., and BEACH, B. A.
1937. THE PRODUCTION OF MILK OF ABNORMAL COMPOSITION BY ANIMALS FREE FROM UDDER STREPTOCOCCI. Jour. Agr. Res. 54: 199–220.
(4) ——— and BEACH, B. A.
1939. A FURTHER STUDY OF THE PRODUCTION OF MILK OF ABNORMAL COMPOSITION BY COWS FREE FROM UDDER STREPTOCOCCI. Jour. Agr. Res. 58: 543–556.
(5) JOHNS, C. K., and HASTINGS, E. G.
1938. THE RELATION BETWEEN BACTERIAL NUMBERS AND BIOCHEMICAL VALUES IN MILK FROM STREPTOCOCCUS-FREE QUARTERS. Canad. Jour. Res. 16 (sect. D): [15]–30, illus.
(6) LITTLE, RALPH B.
1939. THE EFFECT OF ADVANCED LACTATION IN THE COW ON THE PHYSICAL AND CHEMICAL COMPOSITION OF THE MILK. Jour. Dairy Sci. 22: 689–695.
(7) MEIGS, EDWARD B., CONVERSE, HENRY T., BURKEY, LLOYD A., ROGOSA, MORRISON, and SANDERS, GEORGE P.
1938. THE RELATION OF MILKING MACHINES TO THE INCIDENCE OF MASTITIS. 11 pp., illus. [mimeographed.] Paper presented at 33d meeting Amer. Dairy Sci. Assoc., Colombus, Ohio. (Abstract) Jour. Dairy Sci. 21 (5): 165–166.
(8) MUNCH-PETERSEN, E.
1839. BOVINE MASTITIS, SURVEY OF THE LITERATURE TO THE END OF 1935. 272 pp. Imp. Bur. Anim. Health. Weybridge, Surrey, England.
(9) PETERSON, E. H., and HASTINGS, E. G.
1939. A STUDY OF THE POSSIBLE RELATIONSHIP BETWEEN NONSPECIFIC MASTITIS AND STREPTOCOCCUS INFECTION OF THE BOVINE UDDER. Cornell Vet. 29: 11–24, illus.
(10) ——— HASTINGS, E. G., and HADLEY, F. B.
1938. THE PATHOLOGY OF NONSPECIFIC MASTITIS AND CONSIDERATION OF POSSIBLE ETIOLOGICAL AGENTS. Cornell Vet. 28: 307–324, illus.
(11) PLASTRIDGE, W. N., ANDERSON, E. O., WILLIAMS, L. F., and WEIRETHER, F. J.
1939. INFECTIOUS BOVINE MASTITIS. 7. CHARACTERISTICS OF UDDER STAPHYLOCOCCI. Conn. (Storrs) Agr. Expt. Sta. Bul. 231, 60 pp.
(12) PORCHER, CH.
1923. DU CHLORURE DE SODIUM DANS LE LAIT. Lait 3: [11]–21.
(13) RUSSELL, H. L., and HOFFMANN, CONRAD.
1907. DISTRIBUTION OF CELL ELEMENTS IN MILK AND THEIR RELATION TO SANITARY STANDARDS. Wis. Agr. Expt. Sta. Ann. Rpt. 24: 231–253.
(14) SHOPE, RICHARD E.
1939. COMPLEX INFECTIONS. Arch. Path. 27: 913–932.

INFLUENCE OF THE PHYSIOLOGICAL AGE OF THE PEA PLANT ON ITS RECOVERY FROM APHID DAMAGE[1]

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INTRODUCTION

During 1938, 335 lots of peas comprising 275 varieties varying in earliness of maturity were tested for resistance to the pea aphid (*Illinoia pisi* Kalt.). The aphid population, which had increased rapidly and reached destructive numbers during the test, was suddenly destroyed, apparently by fungi. The extent of recovery of the plants after the disappearance of the aphids appeared to be markedly influenced by the stage of maturity or "physiological age" of the respective varieties at the time of the infestation. The results of an analysis of this relationship is presented.

MATERIALS AND METHODS

The peas were planted May 1 in plots consisting of nine rows 6 feet long and 8 inches apart. Each plot was 2 feet from the adjacent plots in each direction. The seeds were planted by hand, about 1½ inches apart in the row. All plots were infested artificially on May 23 from an aphid stock previously reared in a greenhouse. This infestation, applied 2 weeks before the anticipated peak of spring migration from alfalfa, was believed to be sufficient to seriously injure all except the most resistant varieties. The identification of the resistant varieties, in this way, was anticipated.

RISE AND DECLINE OF THE APHID POPULATION

Cool and rainy weather kept the aphid population low until early June, when conditions changed and the aphid counts showed rapid increases. On June 22, an average of 2,575 aphids was recorded for each 360° sweep with an 11-inch net. This number marked the peak of aphid infestation during the season. Coincident with several warm rains between June 20 and 25, an outbreak of fungous disease occurred among the aphids, and the rapidity and completeness of their disappearance was unusual. Net sweepings on June 30 showed an average of only 39 aphids per sweep. The aphids had reached damaging numbers by June 14 and continued to cause injury until about June 24, making a total damage period of about 10 days. A graph of the 1938 aphid population on peas is shown in figure 1.

NATURE OF INSECT DAMAGE TO PLANTS

The effect of the infestation was fairly uniform throughout the plots. The aphids concentrated upon the apical parts of the plants, injuring the tissue produced just before and during the time of heaviest infes-

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tation. These young and succulent vegetative parts became permanently shriveled and malformed. Well-formed pods were stunted and deformed, while very young pods and blossoms were blighted and fell. Several varieties dropped their blossoms even before the attack became severe. Others held their young pods until the pedicels had lengthened, the pods then falling. Still other varieties retained their pods throughout the infestation, the pods becoming twisted and dwarfed and producing at most, one or two peas. Spindly-stemmed varieties such as Alaska and Wisconsin Early Sweet suffered most, the growing tips being killed. Succulent types like Onward and Prince of Wales, although stunted and apparently dead at the apex, recovered.

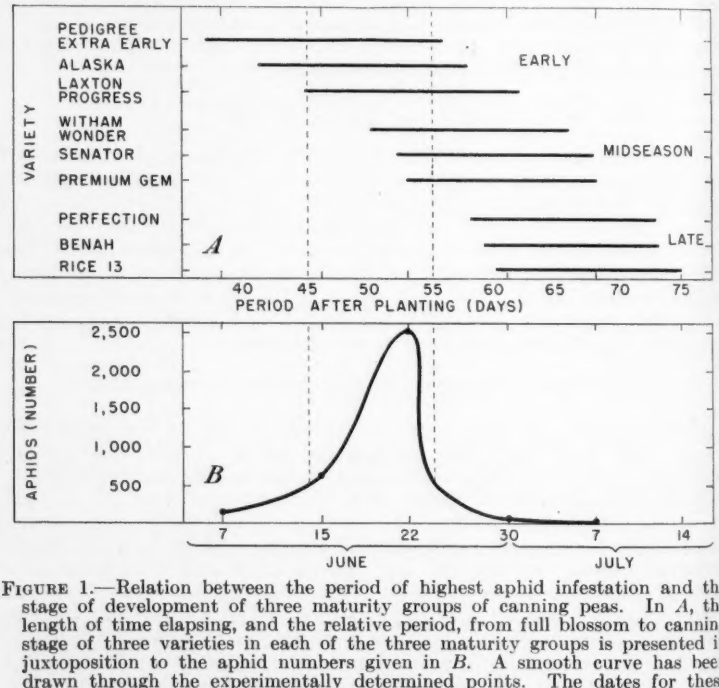

FIGURE 1.—Relation between the period of highest aphid infestation and the stage of development of three maturity groups of canning peas. In *A*, the length of time elapsing, and the relative period, from full blossom to canning stage of three varieties in each of the three maturity groups is presented in juxtoposition to the aphid numbers given in *B*. A smooth curve has been drawn through the experimentally determined points. The dates for these points are given below the figure. Days included between vertical broken lines show period of severe damage.

RELATIVE RECOVERY IN EARLY, MIDSEASON, AND LATE VARIETIES

After the decline of the aphid numbers it was noted that recovery occurred in varying degrees on varieties which could be grouped according to their stage of development at the time of highest aphid population. Since nonrecoveries were predominant among the early varieties while recoveries predominated among the late varieties, it was concluded that resistance was not the principal cause of recovery.

A study was made to determine the incidence of recoveries and nonrecoveries among varieties of different maturity ranges. The varieties were grouped into four classes according to the number of days required to reach the canning stage from date of planting. Varieties requiring 55 to 65 days were grouped as early, those requiring 65 to 75 days as midseason, both early and late, and those requiring 75 to 85 days as late. A summation of the varieties into these maturity groups and their reaction after injury is presented in table 1.

TABLE 1.—*Nonrecoveries, partial recoveries, and recoveries from aphid infestation among 335 lots of peas comprising 275 varieties maturing at different times*

Blossom date	Maturity range	Lots showing—		
		Nonrecovery	Partial recovery	Recovery
Before June 15	Early	82	12	8
June 15–25	Midseason:			
	Early	16	14	18
	Late	2	6	86
After June 25	Late	1	3	87
Total		101	35	199

DISCUSSION OF PEA PLANT BEHAVIOR

Peas usually develop new nodes at fairly regular intervals, and without great variation in time between varieties. Alaska and Perfection varieties, if planted together, will develop node No. 10, for example, at about the same date. Alaska normally produces its first blossom at node 10, however, and bears its main crop of pods on nodes 10 to 15. Perfection, the later variety, produces its crop on nodes 16 to 22. When the first pods set have matured, growth ceases and the plants die soon after the seed is ripe.

The period of aphid damage lasted from about the forty-fifth to the fifty-fifth day after planting. In terms of vine growth, it occurred while the parts produced from nodes 13, 14, and 15 were tender and succulent. Aphids were attracted in greatest numbers to these parts and the damage was great. Early varieties, blossoming on nodes 9 to 12, depending on the variety, had set from two to six pods before damage was done (fig. 2, *A*).

In the midseason varieties, normally producing their first blossoms on nodes 13, 14, or 15, the first blossoms were blasted. Only a few varieties which may have been somewhat resistant were able to set one or two pods during the infestation (fig. 2, *B* and *C*). Late varieties which blossom after the fifteenth node escaped both blossom and pod injury. The aphids disappeared before the first blossoms were produced (fig. 2, *D*). The time in days from blossoming to canning for representative varieties of the three general maturity groups is thus correlated with the period of aphid damage in figure 1.

CORRELATION OF PLANT GROWTH STAGE AND RECOVERY

Before the aphid population disappeared, early varieties had produced some well-developed seed. These plants were physiologically nearing maturity although no older in point of time from planting or

FIGURE 2.—Line drawings made over photographs taken July 7, 1938. The effect of the severe aphid damage upon the various maturity groups is shown between the heavy horizontal lines. *A*, Laxton Progress, early; *B*, Witham Wonder, early midseason; *C*, Premium Gem, late midseason; *D*, Benali, late. Extent of growth above upper line indicates amount of recovery.

node development than other varieties. Canning peas do not ordinarily regenerate after they have reached maturity. After the aphids had been destroyed there was little or no new growth in this group of peas.

Midseason varieties had reached from early to full bloom during the period of damaging aphid numbers. For the most part, all blossoms and young pods present were so injured at this time that they fell from the vines. The amount of growth produced after the period of damage was apparently limited by the age of the first surviving pods produced. If a variety retained one or two well-developed, even if malformed, pods during the infestation, there was little regeneration. If a variety had set no pods prior to the infestation, new growth continued until the first-formed postinfestation pods became mature. Several varieties put out adventitious shoots from nodes immediately below the injured area (fig. 2, *C*). Those varieties that were able to produce and hold some pods during the infestation, actually produced a smaller yield than those that were unable to produce pods during the damage period. Those midseason varieties that lost all of their reproductive parts as a result of the aphid attack regenerated when the aphids were destroyed. Blossoms were produced at later nodes and an apparently normal set of pods followed.

Late varieties, although retarded by two or three shortened and weakened internodes, soon outgrew the damage. Few blossoms had been produced by the late varieties at the period of damage, and those that had been produced were blasted and fell from the plant. After the aphids had disappeared, the relatively uninjured growing tips quickly resumed growth. When the first blossom nodes were reached, blossoming began and the plants produced an apparently normal crop of peas.

CONCLUSIONS

From these studies it has been concluded that the instance described above is a clear case of escape and that the ability of varieties in certain maturity groups to produce a satisfactory crop of peas is not evidence of resistance Escape, such as described above, may not be depended upon to protect crops from insect injury and is therefore of little value to the plant breeder or entomologist who is trying to produce resistant varieties. The phenomena described are of significance in two outstanding respects: (1) They indicate that comparative yield alone may not be depended upon as a measure of the ability of the plant to resist insect attack, and (2) that the physiological age of the plants, not resistance, appears to determine the comparative ability of the various maturity groups to recover after an aphid attack has stopped short of actual destruction of some of the varieties.

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MINIMUM LETHAL DOSE OF SELENIUM, AS SODIUM SELENITE, FOR HORSES, MULES, CATTLE, AND SWINE[1]

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INTRODUCTION

Following the discovery of selenium in numerous plants grown in certain areas of the north-central Great Plains and its identification as the cause of so-called alkali disease of livestock in those areas, a number of experiments have been undertaken to test its toxicity. Most of this work was designed to study the effect of continued feeding of small doses of selenium in various forms to small experimental animals. The disease produced in this way was of the subacute or chronic type. In a few studies, however, large single doses of selenium have been administered, resulting in the acute type of poisoning. It appears that only small laboratory animals have previously been used for this work. Franke and Moxon,[3] using rats, found that the minimum fatal dose (smallest dose that killed 75 percent or more of the animals in 48 hours) of selenium, in the form of sodium selenite, injected intraperitoneally was 1.5 mg. per pound of body weight. Of sodium selenate, the minimum fatal dose was found to be 2.5 mg. per pound of body weight. Smith, Stohlman, and Lillie,[4] using rabbits, found that the fatal dose of selenium, in the form of sodium selenite or selenate, when administered orally was 1.5 to 2.0 mg. per pound of body weight. No reference has been found of any such experimentation with the larger domestic animals.

In order to obtain information on this point, experiments were carried on in 1934 and 1938 at the Animal Disease Station, Beltsville, Md., to determine the minimum lethal dose of selenium for horses, mules, cattle, and swine. This work also afforded an opportunity to observe any symptoms and gross pathological changes which might result from acute selenium poisoning and how the element was distributed in the body tissues. The results of the experiments are reported in this paper.

EXPERIMENTAL PROCEDURE

The number and weights of the different species of animals used in the experiments, the dosages given, and the methods of administration appear in table 1. The animals were normal as far as could be ascertained by clinical examination. Some of the horses and mules, however, had been included in infectious-disease experiments before being used in this work. Horse 775 had been given 5 cc. of a 24-hour culture of *Pasteurella bubaliseptica* a few weeks before it received sele-

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[2] The writers are indebted to H. C. Dudley, formerly of the Bureau of Chemistry and Soils, for chemical analyses, for selenium, of the tissues, milk, and urine of several experimental animals. Acknowledgment is also made to G. T. Creech of the Bureau of Animal Industry for histopathological examinations.

[3] FRANKE, K. W., and MOXON, A. L. A COMPARISON OF THE MINIMUM FATAL DOSE OF SELENIUM, TELLURIUM, ARSENIC, AND VANADIUM. Jour. Pharmacol. and Expt. Ther. 58: 454–459. 1936.

[4] SMITH, M. I., STOHLMAN, E. F., and LILLIE, R. D. THE TOXICITY AND PATHOLOGY OF SELENIUM. Jour. Pharmacol. and Expt. Ther. 60: 449–470. 1937.

nium. Horses 875 and 1003 and mules 991 and 1033 had been exposed to infectious anemia virus. Horse 1009 had been used in several infectious-disease experiments, whereas no previous experimentation had been done on horse 764 or on mule 1035. Although no records were available concerning the ages of the horses and mules, it was evident that they were 8 years of age or more.

TABLE 1.—*Summary of experiments to determine the toxicity of single doses of selenium in the form of sodium selenite administered orally to horses, mules, cattle, and swine*

Species and No.	Live weight	Dosage of		Method of administration	Selenium as sodium selenite per pound of body weight	Result
		Sodium selenite	Water			
Horse:	*Pounds*	*Grams*	*Cubic centimeters*		*Milligrams*	
775	1,185	14.22	100	Drench	5.50	Death in 24 hours.
764	970	9.70	500	Stomach tube	4.60	Death in less than 24 hours.
875	1,435	11.48	500	do	3.65	Do.
1009	1,450	6.34	500	do	2.00	Death in 26 hours.
Mule:						
1033	1,227	4.02	500	do	1.50	Death in 36 hours.
1035	925	3.03	500	do	1.46	Death in about 36 hours.
991	850	2.79	500	do	1.50	Very sick.
Horse 1003	1,110	2.98	500	do	1.23	Sick.
Calf 1	66	1.32	50	Drench	9.15	Death in 6 hours.
Cow:						
1729	1,050	14.71	85	do	6.40	Death in about 48 hours.
1912	1,360	16.32	95	do	5.10	Death in about 30 hours.
1914	705	7.05	75	do	4.57	Very sick.
1839	710	5.00	50	do	3.20	No effect.
Pig:						
4282	53	1.20		Feed	10.30	Death in 72 hours.
4754	130	2.28	35	Drench	7.90	Paralyzed; killed after 18 days.
4737	100	1.31	35	do	6.00	Sick.
4730	120	1.57	20	do	6.00	Do.
4683	120	1.05	20	do	4.00	Do.
4703	120	.52	15	do	2.00	Slightly sick.
4746	150	.33	15	do	1.00	No effect.

Two of the cows, Nos. 1914 and 1839, were in the first lactation period and were between 3 and 4 years old, whereas the other two were several years older. The calf was 5 days old. The pigs ranged in age from 4 to 6 months. None of these animals had been exposed directly to any known sources of infectious disease.

Selenium was given orally to each animal, in the form of sodium selenite, in the feed, as a drench, or by stomach tube. All the dosages were calculated on the basis of body weight. Since no information was available on what constituted a toxic or lethal dose for large animals, a fairly large quantity was given in the beginning and was reduced progressively in subsequent tests on other animals until symptoms of acute poisoning were obtained. The animals of each species (horses and mules being grouped together) are listed in table 1 according to size of the dose, with one exception.

EXPERIMENTAL RESULTS

HORSE 775

Horse 775, which was dosed in the afternoon, was depressed, weak, and trembling on the following morning, and its breath had a distinct odor resembling that of garlic. Death occurred in the afternoon of the same day.

On post mortem few lesions were found. The lungs were expanded and marked with the outline of the ribs, and although the interlobular

connective tissue was easily observed no emphysema was present. The heart contained black, coagulated blood. Black hemorrhages about 1 cm. in diameter were found along the coronary groove and in the ventricles. The intestinal tract was reddened and filled with bloody fluid.

HORSE 764

Horse 764 was treated about 1:30 p. m., at which time it appeared normal in all respects and its temperature was 99.2° F. About 10 a. m. on the following day the temperature had risen to 102°. At this time there were slight spasms of the neck and face muscles and grinding of the teeth. The eyes were dilated and staring. There was profuse sweating with distension of the cutaneous blood vessels, although the pulse was very weak. The nostrils were expanded and the respirations were labored, with accessory intercostal breathing. The animal died about 1 hour later.

On post mortem a few small hemorrhages were found on the visceral pleura and the surface of the lungs. There were a few petechiae on the pericardium, and in the heart itself multiple hemorrhages occurred under the endocardium, which extended slightly into the myocardium. The liver showed slight diffuse fatty degeneration. A few hemorrhages, as well as severe gastritis, were found in the stomach. There was marked enteritis. A few subcapsular hemorrhages were noted on the spleen. No marked changes were present in the kidneys, but the bladder showed a diffuse cystitis.

Chemical analysis of the tissue of this animal was made for selenium. The results appear in table 2.

TABLE 2.—*Selenium content of body parts and fluids of animals that received single doses of selenium*

[Results given in parts of selenium per million, based on weight of samples of body parts and fluids taken on post mortem]

Body part or fluid	Selenium content[1] of indicated body part or fluid of—										
	Horse No.			Mule No.		Calf No.	Pig No.				
	764	875	1009	1033	1035	1	4282	4703	4730	4737	4754
	Parts per million	*Parts per million*	*Parts per million*	*Parts per million*	*Parts per million*	*Parts per million*	*Parts per million*	*Parts per million*	*Parts per million*	*Parts per million*	*Parts per million*
Bile						5.0	2.0				
Blood	2.0	2.5		0.8	2.0						2.5
Blood and lymph						27.0					
Bone (left humerus)							T.				
Bone (rib)							T.				
Bone marrow (right femur)							5.0				
Brain	.2	.4									.3
Colon and contents							50.0				
Feces	8.0				10.0						
Gall bladder											2.0
Heart	.4	.4	0.5	.3	1.0	16.0	.05	0.05	0.1	0.5	.1
Hide and hair							.0				
Hoof	.2	.2									2.0
Intestines (small) and contents						14.0					
Intestines (small), tissue							5.0				
Kidney	10.0	8.0	10.9	2.0	6.0	18.0		.2	.5	.2	.3
Liver	5.0	8.0	2.0	1.5	2.0	25.0	2.5	.1	.2	.2	1.0
Lung	1.5	1.5	.7	.5	1.0	8.0	1.0	.05	.5	.2	.2
Muscle, leg	.3	.5					T.				.2
Muscle, rib							T.				
Spinal cord											.1
Spleen	.4	.5	.5	.4	1.0	20.0	10.0	.05	.05	.1	.2
Stomach tissue	20.0	62.0									.5
Urine			1.4	20.0	4.0						4.0

[1] T.=trace.

HORSE 875

Horse 875, which was dosed at the same time as horse 764 (about 1:30 p. m.), was found dead at 8 o'clock the following morning.

On post mortem, the carcass was found to be very fat and there were multiple subcutaneous hemorrhages. In the lungs there were small hemorrhages of the visceral pleura. Numerous petechiae were found on the pericardium, and there were subendocardial hemorrhages in the ventricles. In the stomach there was marked hemorrhagic gastritis. The liver was marbled with fatty degeneration. The spleen contained a few subcapsular hemorrhages of varying size. The kidneys appeared more or less normal, but an ulcerative cystitis was present in the bladder.

Chemical analysis of the tissue of this animal also was made for selenium. The results are given in table 2.

HORSE 1009

Horse 1009 was treated at 11:30 a. m. At 4 p. m. of the same day, its temperature was 100.6° F., and the animal seemed normal. At 9 a. m. the following day, the temperature was 101.0°. The animal was breathing very hard, and the pulse was rapid. An hour and a half later the horse was down, the temperature was 103.6°, and tonic spasms were noted. Death occurred at 1:10 p. m., or about 26 hours after the selenium had been given.

On post mortem, the blood was found to be almost black in color and had not coagulated 1½ hours after death. No lesions were observed in the lungs or in the liver. There were ecchymotic hemorrhages along the coronary groove of the heart and subendocardial hemorrhages in the ventricles. The kidneys were friable, and there were hemorrhages in the cortex.

Chemical analysis of the tissue of this animal was made for selenium. The results are given in table 2.

MULE 1033

Mule 1033 was treated about 1 p. m. At 4 p. m. of the same day, the temperature was 102.2° F. The next morning, the temperature was 101.0°, at which time the mule was breathing hard, with nostrils distended. The pulse was very weak. Feed and water were refused. A blood sample collected at this time was black but was not hemolyzed. In the afternoon the temperature was 100.2°. The animal was found dead on the morning of the second day.

On post mortem the lungs appeared more or less normal, except for a few small black hemorrhages under the visceral pleura. There were also many black ecchymotic hemorrhages in the mediastinum at the bifurcation of the bronchi and under the parietal pleura in the intercostal spaces. The heart contained numerous small black hemorrhages along all the coronary vessels as well as on the auricles. In the ventricles a few subendocardial hemorrhages were found. The blood was black and did not coagulate readily. There was some fatty degeneration of the liver. In the spleen, a few subcapsular hemorrhages were found. Areas of acute enteritis appeared along the small intestines, and the blood vessels were distended with black blood. There was an acute nephritis as well as an acute catarrhal cystitis. The urine was coffee colored and contained numerous gelatinous clots.

Chemical analysis of the tissue of this animal was made for selenium. The results are given in table 2.

MULE 1035

Mule 1035 was treated about 11:15 a. m. At 4 p. m. of the same day the animal's temperature was 101.5° F., pulse 76, and the respirations were fast. It had no appetite and appeared sluggish. On the following day, the temperature was 101.6°, the pulse was weak, and the respirations were fast and labored. The animal had not eaten or drunk since the previous day. At 4 p. m. the pulse was 90, temperature was 101.5°, and the respirations were fast and very labored. It was found dead on the morning of the second day.

On post mortem there were large subcutaneous hemorrhages over the ribs. The lungs were congested. Numerous small hemorrhages were present on the fat of the heart, and very large hemorrhages were found along the coronary groove and under the endocardium of the left ventricle. The blood was black but had not coagulated. There was a fibrinous peritonitis on the lower part of the diaphragm and subperitoneal hemorrhages under the processes of the lumbar vertebrae. Numerous fibrinous tags were present on the surface of the liver, which was marbled with large areas of fatty degeneration. Very few petechiae were observed on the spleen. There were hemorrhagic enteritis and large black hemorrhages in the folds of the mesentery. The kidneys were soft and friable.

Chemical analysis of the tissue of this animal was made for selenium. The results are given in table 2.

MULE 991

Mule 991 was treated at 1 p. m. The next morning the temperature was 99.4° F., and the pulse was weak. The respirations were labored and fast, and the nostrils were distended. It did not eat and drank little water. A blood sample drawn at this time was black but not hemolyzed. In the afternoon the temperature was 102.2°, but the general condition remained unchanged. The animal drank a considerable quantity of water on the second morning but did not eat. The temperature was 100°. The breathing, however, seemed normal although the pulse remained weak. From this time on the mule improved, and 7 days after the selenium was given it was eating and drinking and appeared entirely normal.

Chemical analysis of the blood sample showed a selenium content of 1 p. p. m.

HORSE 1003

Horse 1003 was treated at 12:45 p. m. The animal was not eating or drinking on the following day, and there was some diarrhea. It was breathing hard. A blood sample collected on the morning of the second day was very black. At this time, the horse was eating hay and drinking water again and the respiration had returned to normal. There was no change in the temperature at any time, and the horse remained normal for the remainder of the period of observation.

Chemical analysis of the blood sample showed a selenium content of 0.5 p. p. m.

CALF 1

Calf 1 was treated about 9:30 a. m. and died about 3:30 p. m. the same day. At this time there was a discharge from the mouth and a profuse diarrhea. The animal was not autopsied. Chemical analysis of the tissue of this animal was made for selenium. The results are given in table 2.

COW 1729

Cow 1729 was lactating and not pregnant. It was treated about 9:30 a. m., and in the afternoon of the same day was salivating profusely and appeared very dull. It did not eat or drink. The condition was about the same on the following morning, except that there was no salivation. The animal was down in the afternoon, the breathing was labored, the eyes were dull, and the extremities were cold. The breath had a marked garlic odor, and the feces a peculiar fetid odor. The animal was found dead on the second morning.

On post mortem the lymph glands throughout the body were hemorrhagic. Extensive pleural adhesions were found in the thoracic cavity, but the lungs appeared relatively normal. In the heart there were numerous subepicardial hemorrhages which were particularly marked on the left ventricle. Three to four liters of straw-colored fluid was present in the peritoneal cavity. There were numerous hemorrhages about 1 cm. in diameter throughout the liver, in one lobe of which was a circumscribed abscess, about the size of a baseball, containing white fluid pus. A few subcapsular hemorrhages were found in the spleen. Both kidneys showed an acute nephritis with a few small hemorrhages under the capsules, but the urine appeared normal.

A milk sample taken on the first day after ingestion of the sodium selenite contained 0.02 p. p. m. of selenium. On the second day at the time of death the milk contained 0.04 p. p. m. and the urine 5 p. p. m. of selenium.

COW 1912

Cow 1912 was dry and carrying an 8-month-old fetus. It was treated in the morning, and death occurred late in the afternoon of the following day. On post mortem few lesions were found.

COW 1914

Cow 1914 was lactating and was rather thin at the time it was treated. The next day the animal took little feed or water, and milk production was greatly reduced. On the second day the cow was down practically all the time and had stopped giving milk. It did not eat or drink during this time. Two days later, however, it was eating hay and drinking again and also giving a small quantity of milk. From this time until the cow was autopsied 5 days later it ate only hay, drank little water, and seemed to lose slightly in weight. Milk production did not return to the pretreatment level. Post-mortem findings were entirely negative.

COW 1839

Cow 1839 was lactating and in good condition when treated. The animal was observed for the next 20 days, and no clinical symptoms of selenium poisoning were shown at any time. There was no evident

impairment of the appetite for either grain or hay, the usual quantity of water was taken, and milk production remained the same as before treatment. However, the feces had a peculiar fetid odor on the second day after the selenium was given, and on the following day a somewhat similar odor was noted in the milk at the time it was drawn. On post mortem, 20 days later, the animal appeared entirely normal.

On the fifth and sixth days after ingestion of the selenium the milk was found to contain 2 and 3 p. p. m., respectively. The selenium content of the urine on the sixth day was 3 p. p. m.

PIG 4282

Pig 4282 was given dry sodium selenite mixed with a little moistened mill feed, and the mixture was eaten readily. Few clinical symptoms of selenium poisoning were noted during the next 2 days, but the animal was found dead on the morning of the third day.

On post mortem the carcass was in good condition, the hair was firm, but the skin was slightly reddened. The cutaneous blood vessels were filled with dark, coagulated blood. The pleural cavity contained about 150 cc. of clear reddish-yellow fluid. The trachea and bronchi were filled with foam, and some pneumonic changes were found in the lungs. The heart contained a large quantity of black blood, and there were a few hemorrhages along the coronary vessels. About 200 cc. of yellowish fluid was present in the stomach, and the mucosa of the fundic portion was slightly reddened. The intestines appeared practically normal, but the mesentery contained numerous large dark hemorrhagic areas and the mesenteric lymph glands were black.

On histological examination there was extensive congestion in the capillary blood vessels and thrombi in a number of the larger vessels of the liver. Most of the liver cells showed extreme cloudiness with fatty changes, together with cell vacuolation, and many of these cells also contained deposits of bile pigment. Few normal liver cells were observed, but there was no perceptible increase in the interstitial structure of the organ.

In the kidney there was marked capillary engorgement with slight hemorrhages and also thrombi in several of the larger vessels. Changes in the tubular epithelium varied from cloudiness and loss of cell nuclei to complete destruction of the cells. Groups of tubules were seen in various areas which exhibited more advanced degenerative changes than in other parts of the section. Fatty changes with cell vacuolation and exfoliation of the epithelium in many tubules were particularly noticeable in these areas. Varying amounts of pigment were observed in this section.

There was blood engorgement, particularly in the larger vessels of the spleen, and a slight edema. The interstitial tissue appeared to be somewhat increased.

The changes in the lungs were typical of catarrhal pneumonia, such as thickening of the alveolar walls, cellular infiltration, and sloughing of the bronchial epithelium.

Practically all the vessels of the heart muscle were engorged with blood. The muscle fibers showed cloudiness with beginning fatty changes. Rather heavy deposits of greenish-yellow pigment (bile) were observed, particularly along the course of the blood vessels.

Chemical analysis of the tissue of this animal was made for selenium. The results are given in table 2.

PIG 4754

Pig 4754 was treated about 9:25 a. m. Within the next 5 hours, the pig had vomited four times. On the following day the animal was down, shivering, and had a profuse diarrhea. Post mortem was made 18 days later. During that period the pig was unable to move its legs, although it appeared fully conscious and was able to eat when grain was offered.

On post mortem relatively few lesions of any consequence were found. There was some fatty degeneration of the liver and a few hemorrhages in the cortex of the kidneys. Chemical analysis of tissue for selenium is given in table 2.

PIG 4737

Pig 4737 was treated at the same time as pig 4754. On the following day the animal was extremely sick, although it was not observed to vomit at any time. This pig lay down most of the time, ate little, shivered, squealed, and ground its teeth a great deal. On the fourth day the animal was able to move about and appeared somewhat better, and by the fifth day it seemed to have returned practically to normal and was eating again.

On post mortem 2½ months later no lesions were found. Chemical analysis of the tissue of this animal was made for selenium. The results are given in table 2.

PIG 4730

Pig 4730 was treated in the morning. Within the next 24 hours it vomited greenish material several times. At the same time there was considerable depression, no appetite, and disinclination to move about. From this time on the animal recovered gradually until 1 week later it appeared to be entirely normal.

On post mortem, 2½ months later, the only lesions noted were subendocardial petechiae in the left ventricle. Chemical analysis of the tissue of this animal was made for selenium. The results are given in table 2.

PIG 4683

Pig 4683 was treated in the morning. Although this animal did not vomit, on the following day it showed symptoms of selenium poisoning resembling those of the other pigs, but not so pronounced. Two days later it began to eat and within 5 days returned to normal.

PIG 4703

Pig 4703 was treated in the morning. The animal was slightly sick for the next 24 hours, as evidenced by lack of appetite and general sluggishness. The following day the animal appeared to have recovered fully.

On post mortem, 2½ months later, a slight pericarditis constituted the only gross lesion found. Chemical analysis of the tissue of this animal was made for selenium. The results are given in table 2.

PIG 4746

Pig 4746 never showed any clinical symptoms of selenium poisoning.

DISCUSSION

From the results shown in table 1, it appears that the minimum lethal dose of selenium, when administered by mouth in the form of sodium selenite, has been established within reasonably close limits for horses, mules, cattle, and swine. In addition, it has been demonstrated that there is a distinct difference in the ability of the several species of animals to tolerate various quantities of selenium when given orally in single doses. Enough animals were not included in this work to determine whether there is any difference in susceptibility among individuals within a species.

The minimum lethal dose of selenium for horses and mules was determined with somewhat more exactness than that for the other two species. The first three horses to be treated received 3.6 mg. or more of selenium per pound of body weight. The animals survived for 24 hours or less. When the dose was reduced to about 1.5 mg., one mule out of three survived. The two mules that died after receiving about 1.5 mg. per pound of body weight were very fat, whereas the one that survived was thin as a result of having had infectious anemia (swamp fever). However, typical symptoms of acute selenium poisoning were induced by this dose, and a period of about a week elapsed before the mule was eating and drinking normally again. The minimum lethal dose of selenium for horses and mules was therefore about 1.5 mg. per pound of body weight.

It seemed evident from the results obtained that animals in good condition might be more susceptible to the action of selenium than comparatively thin animals. Therefore, in calculating a dose of selenium for an animal with excess weight, if a dose which merely produces symptoms of selenium poisoning in a thin animal of the same weight is reduced in proportion to the excess weight of the former animal, the same results should be obtained in this animal as in the latter one. To test this assumption a fifth horse (No. 1003) was treated. The animal was very fat, its weight being estimated as 200 pounds in excess of normal for a horse of its size and conformation. The quantity of selenium administered was, therefore, reduced by 18 percent. Typical symptoms of selenium poisoning were produced, from which the animal apparently recovered in about 3 days.

The symptoms of acute selenium poisoning in horses are not particularly characteristic. There is first a lack of appetite and refusal to drink water, and the animal stands quietly in the stall. The pulse is slightly accelerated and the respirations are fast. The temperature may be normal or slightly higher. A few hours before death, the animal stands as though fixed to the floor, eyes staring, nostrils dilated, and the breathing is convulsive, fast, and very labored. The temperature has a tendency to rise, and the pulse is fast and weak or imperceptible in some animals. A blood sample taken at this time is almost black in color, but there is no hemolysis.

That death is due to asphyxiation has been mentioned previously by Beath and coworkers.[5] This explanation seems entirely probable,

[5] BEATH, O.A., EPPSON, H. F., and GILBERT, C. S. SELENIUM AND OTHER TOXIC MINERALS IN SOILS AND VEGETATION. Wyo. Agr. Expt. Sta. Bul. 206, 56 pp., illus. 1935.

both from the symptoms presented in acute cases of poisoning and from the analysis of fractions of whole horse blood by Dudley.[6] In his work it was shown that a "protein-like selenium complex" was present in the erythrocytes and none in the serum, plasma, and fibrin. As a consequence of the formation of this new compound in the red cells and in the presence of sufficient selenium, it is possible that the respiratory capacity of the blood is reduced to a point where death follows. A possible explanation for the difference in susceptibility of the three mules which received 1.5 mg. of selenium per pound of body weight is as follows: A fat animal that is given the same quantity of selenium per pound of body weight as a thin one receives a larger dose in relation to its total blood volume because of the avascularity of fat tissue and has a correspondingly poorer chance for survival. There is a possibility, then, that if the dose of selenium could be calculated in terms of blood volume rather than of body weight, the variation in susceptibility among individuals of the same species would not be so pronounced. Furthermore, oxygen-capacity determinations of the blood would perhaps give even more information on this point because of the apparent affinity of selenium for hemoglobin.

The analysis of tissue from the horses and mules that died of toxic doses of sodium selenite brings out some noteworthy points. The selenium content of the stomach, kidneys, and liver of the animals was high as compared with that of the other tissues examined. The high selenium content of the urine and feces of these fatal cases and of chronic cases[7] shows that large quantities of selenium are eliminated through the kidneys as well as through the intestines. The concentration of selenium in the blood of these animals at death was not so great as in the chronic cases over a period of several months.

In cattle the minimum lethal dose of selenium was found to be between 4.5 and 5 mg. per pound of body weight. This was about three times the quantity required to kill horses. The symptoms of acute poisoning in cattle are similar to those observed in horses, although not so marked.

The analysis of tissue from the calf, which died 6 hours after the administration of 9.15 mg. per pound of body weight, showed a relatively high selenium content throughout the animal.

Swine proved to be even more resistant to the action of single doses of selenium than cattle. One animal (No. 4282), which received 10.3 mg. per pound of body weight, died in 72 hours, and a second pig (No. 4754) was paralyzed, but did not die, after receiving 7.9 mg. Since it had vomited at least four times within 5 hours after dosing, it probably did not retain all the selenium given. Had it received the full effect of the entire dose, it is probable that it would not have survived. Of the two pigs given 6 mg. per pound of body weight, No. 4730 vomited shortly after it was drenched, but there were no indications that No. 4737 had done so. Judging from these results, the minimum lethal dose of selenium for swine is between 6 and 8 mg. per pound of body weight.

[6] DUDLEY, H. C. TOXICOLOGY OF SELENIUM. I. A STUDY OF THE DISTRIBUTION OF SELENIUM IN ACUTE AND CHRONIC CASES OF SELENIUM POISONING. Amer. Jour. Hyg. 23: 169–180. 1936.

[7] Unpublished data.

The symptoms of acute poisoning in pigs consist largely in lack of appetite, sluggishness, and general depression. Diarrhea was observed in one pig, and grinding of the teeth in another.

On post mortem, in cases of acute poisoning, certain common gross changes were found in all four species of animals. Among these were black-colored blood which did not coagulate readily, hemorrhages in the heart (along the coronary vessels and under the endocardium, particularly in the left ventricle), and small subcapsular hemorrhages on the spleen. Less commonly, large hemorrhages were found subcutaneously, in the mediastinum and in the folds of the mesentery. In a few cases there were marked fatty degeneration of the liver and petechiae in the cortex of the kidney.

The analysis of the tissue from pig 4282 shows that the selenium did not get into the muscles, hide, hair, and bones except in very minute quantities during the 3-day interval between ingestion and death. The blood and urine of pig 4754 contained 2.5 and 4 p. p. m., respectively, 18 days after ingestion of sodium selenite equivalent to 7.9 mg. of selenium per pound of body weight. The elimination was well advanced as the selenium content of the body tissues was low. Examination of pigs 2.5 months after ingestion of relatively large doses of sodium selenite showed the nearly complete elimination of selenium.

SUMMARY

Selenium in the form of sodium selenite was given in large single doses to horses, mules, cattle, and swine to determine the minimum lethal dose of the element for these species. It was administered orally in the feed, as a drench, or by stomach tube, the dose being calculated as milligrams of selenium per pound of body weight. Large doses were given at the beginning of each test and the quantity was reduced for other animals until the minimum lethal dose was reached. The experiments were carried on in 1934 and 1938 at the Animal Disease Station, Beltsville, Md.

Five horses and three mules were used. Although all but two had been used previously in infectious-disease experiments, they appeared healthy on clinical examination. The minimum lethal dose of selenium was found to be about 1.5 mg. per pound of body weight.

In the experiments with cattle, one calf and four cows were included. The calf was 5 days old. Two of the cows were between 3 and 4 years of age and the other two were several years older. All these animals appeared healthy clinically and were not known to have been exposed to any infectious disease. The minimum lethal dose for this species was found to be between 4.5 and 5 mg. per pound of body weight.

In the experiments with swine, seven apparently healthy pigs, varying in age from 4 to 6 months, were used. For these animals the minimum lethal dose of selenium was found to be between 6 and 8 mg. per pound of body weight.

HYBRIDIZATION OF AMERICAN 26-CHROMOSOME AND ASIATIC 13-CHROMOSOME SPECIES OF GOSSYPIUM[1]

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INTRODUCTION

Within the last few years a number of workers have attempted to cross cultivated Asiatic 13-chromosome and American 26-chromosome species of cotton (*Gossypium*). Zaitzev (*18*)[2] secured the cross with the 13-chromosome type as female, while Nakatomi (*11*) produced the cross by using the 26-chromosome type as female. Harland (*5*), from several thousand reciprocal crosses, secured 2 hybrids with the 26-chromosome type as female. Feng (*4*) reported having seen 2 natural hybrids between American and Asiatic cottons. He made 1,017 crosses with the Asiatic type as female and secured 1 hybrid. The reciprocal cross gave 5 hybrids from 691 crossed flowers. Feng found that some hybrid seeds contained small embryos and others contained none. When American types were used as female, the capsules dropped within 2 weeks, but when Asiatic species were used as female the capsules reached nearly normal size. Feng determined that pollen tubes reached the base of the styles, and he suggested that the gametes were incompatible. Webber (*16*) made 125 pollinations between cultivated American and Asiatic cottons without securing any crosses. Doak (*3*) attempted to secure crosses between Asiatic and American cottons by pollinating some of the stigmas of American cotton with pollen from an American plant and then pollinating the remaining stigmas of the same flowers with pollen of an Asiatic type. He reported that pollen tubes of the Asiatic species entered the embryo sacs, but no seeds developed.

From a review of work on hybridizing American 26-chromosome and Asiatic 13-chromosome species, it is evident that the few crosses secured resulted from using ordinary methods of hybridization and making hundreds of crosses. It was, therefore, desirable to determine the causes of the high degree of abortion noted in the crosses and to find a way of producing the hybrids at will. It seemed necessary to study pollen germination, pollen-tube growth, fertilization, and the embryology of the hybrids.

RESULTS OF CROSS-POLLINATION

TECHNIQUE

A modification of the technique described by Sears (*12*) was used to study pollen germination and pollen-tube growth. Styles were cut from ovaries 3 to 6 hours after pollination; the epidermis of the fused styles was cut, and the styles were pulled apart; they were then

[1] Received for publication September 25, 1939. Cooperative investigations by the Division of Cotton and Other Fiber Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture, and the North Carolina Agricultural Experiment Station. Part of a thesis problem carried out at Harvard University under the direction of the late Prof. E. M. East.

[2] Italic numbers in parentheses refer to Literature Cited, p. 180.

dropped in boiling 4-percent sodium sulfite solution and boiled 3 to 4 minutes; then washed a few minutes before they were placed in a solution of chloral hydrate (saturated solution diluted with an equal volume of water), to which a few drops of basic fuchsin was added as a stain. After the styles had remained in the dye for several hours, they were placed on slides, the epidermis was removed, and the styles were then pressed with cover glasses. The chloral hydrate solution containing the stain was used as a mounting medium, and the slides were sealed with a mixture of paraffin and gum mastic.

ABNORMAL DEVELOPMENT OF EMBRYO AND ENDOSPERM

It was found that pollen germinated and grew down the styles in reciprocal crosses of American 26-chromosome × Asiatic 13-chromosome species. In both crosses pollen tubes entered more than half the embryo sacs.

Embryos initiated development and seemed for a time to develop as fast as or faster than normal embryos. The development of the endosperm started about the usual time; within 4 to 6 days, however, it usually showed evidence of being abnormal.

Material collected 5 days after pollination of a cross with an American 26-chromosome species as female showed that the ovules were smaller and that the endosperm was usually below normal in development and vigor. The ovules were much undersized 7 days after pollination; the endosperm appeared poorly developed and was clumped toward the center of the embyro sac; but the embyros appeared to be about normal in size. Ordinarily, the capsules aborted 8 to 10 days after pollination, but, as will be explained later, it was possible to make a few capsules continue development. At 19 days after pollination some of the embryos were still growing, although they were much below normal in size. Some ovules of this age had a trace of endosperm.

In the reciprocal cross, with Asiatic 13-chromosome species as female, the story differed in that ovules enlarged at a normal rate. The endosperm started vigorous development, but it soon appeared aberrant and ceased to develop after 7 to 9 days. By 15 days after pollination the endosperm had disappeared. The hybrid embryos grew as fast as or faster than normal ones until the endosperm began to disintegrate, at which time their growth rate was greatly reduced. An examination of ovules 30 days after pollination, the age hybrid capsules usually abort, showed that they contained embryos one-half to 2 mm. in length, whereas normal embryos of the same age are about 8 mm. in length. Division figures with 39 chromosomes were found in acetocarmine smears of 30-day-old hybrid embryos. These hybrid embryos ordinarily are poorly differentiated; they usually have two or more thick lobes in place of cotyledons or consist of undifferentiated tissue.

It became evident that, if a method could be found of developing plants from such minute embryos, hybrids could be secured. As the embryos were hardly more than masses of meristematic tissue, a method was tried that was used by White (*17*) to secure continuous growth of excised tomato roots in culture media made of pure chemical compounds, sucrose, yeast extract, and agar. LaRue (*9*), with White's methods, was able to produce plants by culturing essentially mature embryos and, with some plants, parts of embryos. By germinating

and culturing undersized hybrid seed on nutrient media, Laibach (*8*), Jørgensen (*7*), Skovsted (*13*), and Tukey (*15*) were able to produce hybrids that were difficult or impossible to get by the usual methods.

Hybrid embryos, from crosses of female Asiatic 13-chromosome × male American 26-chromosome species, were dissected from ovules and put on White's culture media after the capsules containing them began to abort. Some of them germinated and formed roots, and the hypocotyls reached a length of about 25 mm., but they had minute deformed structures for cotyledons and never developed further.

RESULTS OF MIXED POLLINATION

METHOD OF POLLINATING

Before the embryology of hybrids between American 26-chromosome and Asiatic 13-chromosome types was studied, it seemed possible that the difficulty in producing the hybrids might result from the fertilization of only a few ovules, which caused the capsules to abort. If this were the trouble, it could be avoided by applying a small amount of pollen from the female plant and then applying an excess of the other type of pollen. Reciprocal mixed pollinations of this type were made.[3]

The method employed to the greatest extent consisted of using a green-leaf 26-chromosome cotton as female, applying 6 to 12 grains of pollen from another plant of the same species that was homozygous for a dominant anthocyanin factor (red leaf), and then covering the stigmas with an excess of Asiatic 13-chromosome pollen. This method was followed in order that hybrids between the species could be easily detected, for interspecies hybrid seedlings would be green and intraspecies hybrids red (*3*).

PRODUCTION OF HYBRID SEEDLINGS

All the first few seeds germinated gave red seedlings. It was noticed, however, that some seeds failed to germinate, so other seeds were examined for embryos before they were planted. Three seeds of about half-normal size were found in one capsule, and the embryos from these seeds were germinated on White's culture media.[4] Acetocarmine

Inorganic compound:	*Grams per 10,000 cc.*
$CaNO_3$	0.98
$MgSO_4$	.38
KNO_3	.80
KCl	.65
KH_2PO_4	.125
$Fe(SO_4)_3$	.025

These compounds are dissolved separately, each in one-thirtieth of the total volume of water (333 cc.) and kept in separate bottles. To make a given amount of media, take four-fifths (of the volume of media) of water, and add one-thirtieth (of the volume of media) of each of the six mineral solutions. To the mixture of the six mineral solutions add 0.6 percent of agar and 2.0 percent of sucrose.

root-tip smears proved them to be hybrids. Further examination of the material produced by mixed pollination showed several minute seeds (fig. 1, *C* and *D*). These seeds were placed on culture media, where several of them germinated (fig. 1, *F* and *G*). They were allowed to grow on the media until they formed roots, and then they

[3] Dr. M. M. Rhoades and Dr. J. M. Webber have informed the writer that the method of mixed pollination was previously known to them, and Jørgensen (*7*) suggested that the application of pollen of a distantly related species to stigmas partly pollinated with pollen of the female parent might be a method of producing haploids.

[4] An adaptation of White's formula is given here for convenience:

were transferred to pots of sterilized soil. It was necessary to invert beakers over these hybrids until they formed new root hairs. They were watered with distilled water and White's solution until they

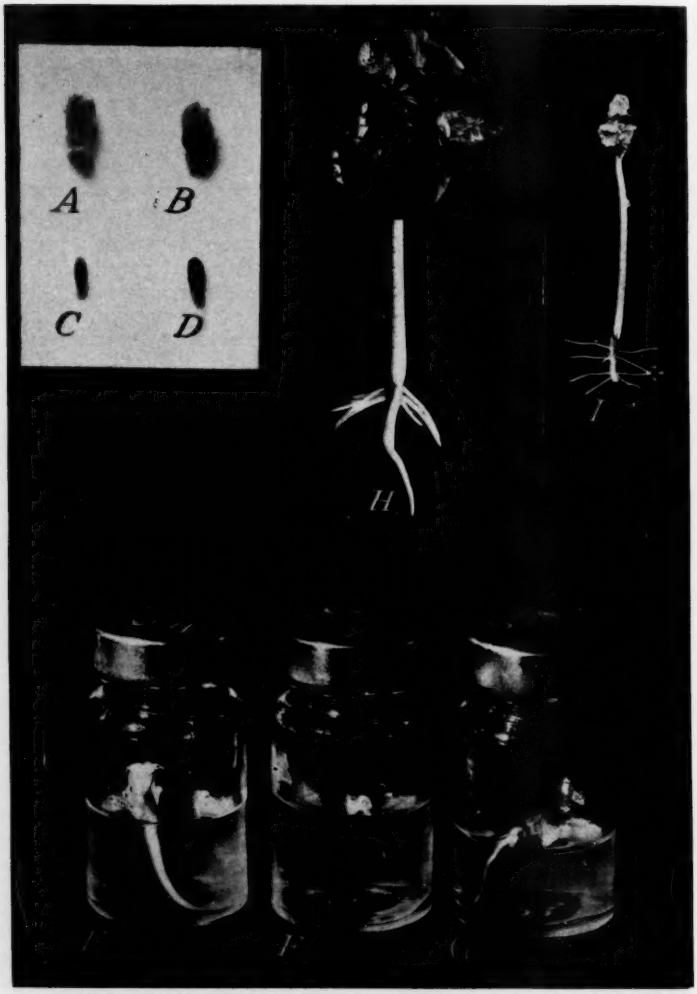

FIGURE 1.—*A* and *B*, Normal seeds of *Gossypium hirsutum; C* and *D*, F_1 seeds of *G. hirsutum* × *G. arboreum* var. *neglectum*, obtained by the method of mixed pollination; *E*, normal seed, and *F* and *G*, hybrid seeds, germinating on culture media; *H*, normal seedling; *I*, comparable hybrid seedling.

showed normal growth. The small size of these hybrids at the time they were transferred to soil is shown by comparing figure 1, *H* and *I*. Skovsted (*13*) secured hybrids of *G. davidsonii* Kellogg × *G. sturtii*

F. Muell. "by removing the seed coats, washing in sterile water, and then sowing in sterile cultures of 5 percent dextrose-agar."

By using the method of mixed pollination and germinating the minute embryos on nutrient media, the following *Gossypium* hybrids were produced:

G. hirsutum L. × *G. herbaceum* L. var. *typicum* Hutchinson and Ghose (*6*).
G. hirsutum L. × *G. herbaceum* L. var. *africanum* Hutchinson and Ghose.
G. hirsutum L. × *G. arboreum* L. var. *neglectum* Watt forma *burmanica* Hutchinson and Ghose.
G. hirsutum L. × *G. arboreum* L. var. *cernuum* Hutchinson and Ghose.
G. hirsutum L. × *G. arboreum* L. var. *typicum* forma *bengalensis* Hutchinson and Ghose.
G. barbadense L. × G. *herbaceum* var. *typicum*.

The apparently normal seeds from about 70 capsules, obtained by the method of mixed pollination, were planted in soil. Two of the seeds produced green plants, while other seeds from the same capsules gave red plants. One of the green plants was a hybrid, the other was a normal American 26-chromosome plant. The natural hybrids reported by Feng (*4*) and Longley (*10*) doubtless resulted from mixed pollinations.

One other cross, *G. religiosum* L. × *G. arboreum* var. *neglectum*, of an American 26-chromosome × an Asiatic 13-chromosome species was secured from a seed of normal size. In this case, *G. religiosum* was used as female and only pollen of *G. arboreum* var. *neglectum* was used.

If the mixed pollinations are made under optimum conditions more than 75 percent of the capsules reach maturity. Some of them fail to have seeds with hybrid embryos, while others have from one to four minute seeds with hybrid embryos. In the present work hybrids were produced at the rate of one from about each five capsules in which seeds had been produced by the method of mixed pollination.

In relation to the above results of hybridizing American 26-chromosome and Asiatic 13-chromosome cottons, it is of interest to note that an autotetraploid Asiatic cotton with the same number of chromosomes as American cultivated cottons hybridizes easily with American 26-chromosome pollen.

DISCUSSION

Hybrids between cultivated American 26-chromosome and Asiatic 13-chromosome cottons are useful in studying the relationships between the two types and to secure evidence on the origin of the tetraploid American type. Of course only a few hybrids are necessary for such purposes. Recently, however, by the use of colchicine, it has been possible to produce fertile polyploids from the sterile hybrids (*1*). Since there are numerous varieties of American 26-chromosome and Asiatic 13-chromosome cottons, and since slightly different combinations give polyploids with different characteristics, the reason is obvious for the production of numerous hybrids between the types that involve different combinations of varieties.

It is comparatively easy to produce hybrids of American 26-chromosome × Asiatic 13-chromosome species by germinating the minute hybrid seeds on sterile culture media. This technique can also be used to secure plants from nonhybrid seeds that ordinarily fail to survive. For example, two normal plants were grown from tiny seed, motes, from crosses that ordinarily give normal seeds.

Doubtless genetic types could be grown, by using the described method, from abortive seeds that would fail to survive under the usual cultural conditions.

Workers have tried various methods in attempts to produce hybrids between American 26-chromosome and Asiatic 13-chromosome cottons. Desai (*2*) applied substances to the stigma to stimulate pollen germination. The fact that pollen germinates and tubes enter ovules shows that nothing is gained by applying substances to the stigmas. Tanaka (*14*) tried to make capsules with hybrid embryos remain on plants by ringing and wiring the fruiting branches. The study of the embryology of the hybrids shows that such practices can be of little value.

SUMMARY

In reciprocal crosses of American 26-chromosome × Asiatic 13-chromosome cottons, the pollen germinates and pollen tubes enter more than half the embryo sacs. Embryo and endosperm development is initiated, but soon becomes aberrant.

By using the American 26-chromosome type as female and a few grains of pollen from a 26-chromosome type along with an excess of Asiatic 13-chromosome pollen, it is possible to produce minute hybrid seeds. Plants can be produced from the seeds by germinating them on sterile culture media. Hybrids involving six combinations of American 26-chromosome × Asiatic 13-chromosome cottons were produced.

This dependable method of producing hybrids between American and Asiatic cottons will be useful in producing numerous hybrids from which polyploids can be produced.

LITERATURE CITED

(1) Beasley, J. O.
1940. The production of polyploids in gossypium. Jour. Hered. (In press.)

(2) Desai, B. B.
1927. A cross between indian and american cotton. Agr. Jour. India 22: 351–353.

(3) Doak, C. C.
1937. The pistil anatomy of cotton as related to experimental control of fertilization under varied conditions of pollination. Amer. Jour. Bot. 24: 187–194, illus.

(4) Feng, C. F.
1935. Genetical and cytological study of species hybrids of asiatic and american cotton. Bot. Gaz. 96: 485–504, illus.

(5) Harland, S. C.
1932. Fertility in hybrids between new and old world cottons. Nature [London] 129: 398–399.

(6) Hutchinson, J. B., and Ghose, R. L. M.
1937. The classification of the cottons of asia and africa. Indian Jour. Agr. Sci. 7: 233–257, illus.

(7) Jørgensen, C. A.
1928. The experimental formation of heteroploid plants in the genus solanum. Jour. Genet. 19: 133–211, illus.

(8) Laibach, Friedrich.
1929. Ectogenesis in plants. Methods and genetic possibilities of propogating embryos otherwise dying in the seed. Jour. Hered. 20: 201–208, illus.

(9) LaRue, Carl D.
1936. THE GROWTH OF PLANT EMBRYOS IN CULTURE. Torrey Bot. Club Bul. 63: 365–382, illus.
(10) Longley, A. E.
1933. CHROMOSOMES IN GOSSYPIUM AND RELATED GENERA. Jour. Agr. Res. 46: 217–227, illus.
(11) Nakatomi, Sadao.
1931. HYBRIDIZATION BETWEEN OLD WORLD AND NEW WORLD COTTON SPECIES, AND THE CHROMOSOME BEHAVIOR OF THE POLLEN MOTHER-CELLS IN THE F_1–HYBRID. Jap. Jour. Bot. 5: [371]–383, illus.
(12) Sears, E. R.
1937. CYTOLOGICAL PHENOMENA CONNECTED WITH SELF-STERILITY IN FLOWERING PLANTS. Genetics 22: 130–181, illus.
(13) Skovsted, A.
1935. CYTOLOGICAL STUDIES IN COTTON. III. A HYBRID BETWEEN GOSSYPIUM DAVIDSONII KELL. AND G. STURTII F. MUELL. Jour. Genet. 30: [397]–405, illus.
(14) Tanaka, Masao.
1937. THE APPLICATION OF RINGING AND WIRING TO INTERSPECIFIC CROSSES OF THE GENUS GOSSYPIUM. Kyoto Imp. Univ. Col. Agr. Mem. 39: 1–7, illus.
(15) Tukey, H. B.
1938. GROWTH PATTERNS OF PLANTS DEVELOPED FROM IMMATURE EMBRYOS IN ARTIFICIAL CULTURE. Bot. Gaz. 99: 630–665, illus.
(16) Webber, J. M.
1935. INTERSPECIFIC HYBRIDIZATION IN GOSSYPIUM AND THE MEIOTIC BEHAVIOR OF F_1 PLANTS. Jour. Agr. Res. 51: 1047–1070, illus.
(17) White, Philip R.
1934. POTENTIALLY UNLIMITED GROWTH OF EXCISED TOMATO ROOT TIPS IN A LIQUID MEDIUM. Plant Physiol. 9: 585–600, illus.
(18) Zaitzev, G. S.
1927. ARTIFICIAL AND NATURAL ASIATIC-AMERICAN COTTON HYBRIDS. Agr. Jour. India 22: 155–167, 261–268, illus.

A PHYSIOLOGICAL STUDY OF SOFT SCALD IN JONATHAN APPLES [1]

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INTRODUCTION

Although soft scald of apples (*Malus sylvestris* Mill.) has been attributed to a deficiency of oxygen in the surrounding air, the various control measures reported in the literature have not been limited to aeration of the stored fruit. Among the means of control may be mentioned placing the fruit in cold storage without delay (*2*, *8*),[3] maintaining a storage temperature of 36° F. (*11*), coating the fruit with paraffin (*6*) or with a mixture of oil and paraffin (*5*), and short prestorage exposures to partial vacuum, a high temperature, or carbon dioxide (*3*).

Brooks and Harley (*3*) suggested that although the beneficial effects of certain treatments may be due to the elimination of accumulated respiratory products, the effects of "changed metabolism" should not be overlooked. Plagge and Gerhardt (*10*), writing on soggy breakdown of Grimes Golden apples, had already shown that delayed storage produced changes in metabolism by decreasing the acidity and increasing the specific gravity of the juice. The rate of acetaldehyde and alcohol production has been considered an index of the physiology of stored fruits, and recently these substances have been shown to accumulate in apples affected by soft scald (*9*, *12*).

The present investigation was undertaken for the purpose of obtaining a better understanding of the physiological changes produced in apples by treatments known to influence the incidence of soft scald.

MATERIALS AND METHODS

In 1933 and 1934 Jonathan apples were obtained from near Hancock, Md., and in 1935 from near Frederick, Md. The fruit was transported by motortruck from the orchard to the cold-storage rooms at the Arlington Experiment Farm, Arlington, Va., and placed under the following storage conditions employed for the purpose of studying the development of soft scald: Immediate storage at 32° and 36° F.; holding at 65° to 75° for from 3 to 11 days before storing at 32° and 36°; and subjecting the fruit to heating or to carbon dioxide treatment, following the delay and prior to storage at 32°. A bushel of fruit was reserved in each lot for chemical analyses, and a sample consisting of 25 to 30 fruits was removed at regular intervals.

Sugar determinations were made on duplicate 50-gm. samples of pulp or 25 ml. of juice. Analytical methods were identical with those recommended for plants by the Association of Official Agricultural

[1] Recieved for publication October 9, 1939.
[2] The writers are indebted to Charles Brooks and Lacy McColloch, of this Division, for helpful suggestions and assistance in this work.
[3] Italic numbers in parentheses refer to Literature Cited, p. 191.

Chemists (*1*). Sugar was determined either gravimetrically or by the volumetric permanganate method.

Acetaldehyde content of the fruit pulp was determined by a method previously described (*7*). Hydrogen-ion values of the juice were obtained by means of a calomel half cell, a quinhydrone electrode, and a Leeds and Northrup potentiometer. Total acidity was determined by titrating the juice with N/10 sodium hydroxide to pH 7 by means of the above-mentioned hydrogen-ion apparatus.

The "acid index" was computed according to the method of Du Toit and Reyneke (*4*). Actually it represents the ratio of the active acidity to total acidity $\left(\frac{H^+}{H_2}\right)$.

Inasmuch as the 3 years' experiments were replications, only data for 1 year are presented when they are considered typical of all 3 years.

RESULTS

ACETALDEHYDE

In figure 1 are curves showing the acetaldehyde content of Jonathan apples stored in 1934. These curves are typical of the effect of delayed storage, and delayed storage followed by carbon dioxide treatment, on the acetaldehyde content. First it will be noted that the fruit placed immediately in 32° F. storage showed a gradual but slight increase in acetaldehyde content during the storage period. A delay of 6 days at 65° prior to storage at 32° increased the acetaldehyde content from 0.1 mg. per 100 gm. of fresh tissue to 0.4 mg. at once, and at about the middle of the storage period the amount was 1.2 mg. A 2-day treatment with 35 percent carbon dioxide in a closed container increased the acetaldehyde content to 1.4 mg., the highest found in this whole lot, but the midstorage value in this particular lot was lower than the others at that time. By January 1935, the end of the storage period, the acetaldehyde content was not high in any lot, but lowest of all in the lot treated with carbon dioxide. There is no apparent relationship between the acetaldehyde content and the development of soft scald. The results for the fruit stored at 36° are similar to those for fruit stored at 32°.

In table 1 the results for the 1935 crop are reported. This table is included not because the results are in disagreement with the others but because the fruit as a whole produced much smaller quantities of acetaldehyde. In lots 1 and 2 stored immediately at 32° and 36° F. the amounts were 0 for all samples. Nevertheless the effects of delayed storage and carbon dioxide treatment are consistent with those obtained previously. Increase in acetaldehyde content as a result of the 3-day delay is not evident until the October sampling, but the effect of the 6-day delay on acetaldehyde content is immediate. The carbon dioxide treatment produced the highest acetaldehyde content of all the lots. An analysis of the data shows that 32° storage is more conducive to incidence of soft scald than 36° and that a delay of more than 3 days increases the amount of soft scald unless the delay is followed by a carbon dioxide treatment. This is in agreement with earlier reports.

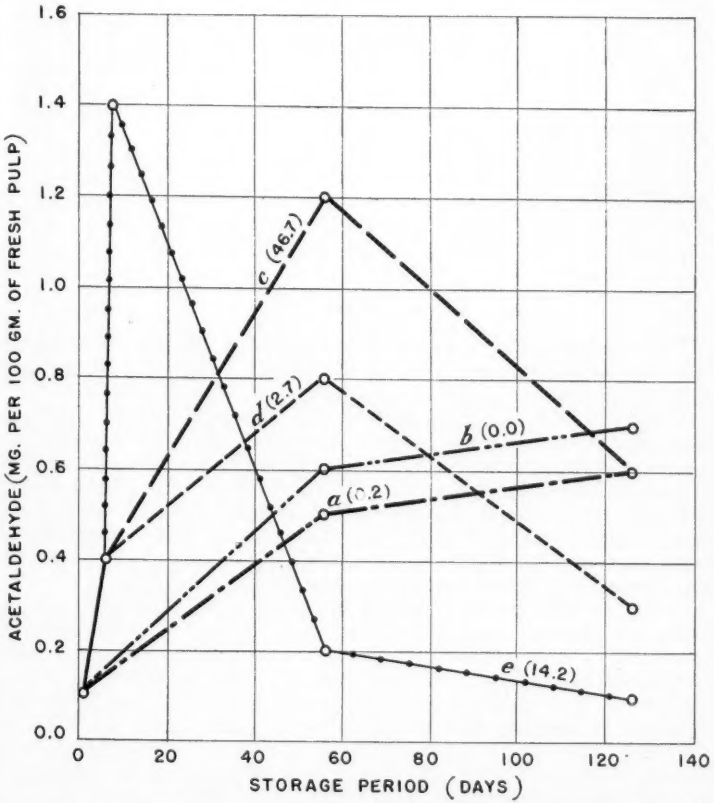

FIGURE 1.—Effect of different storage conditions on the acetaldehyde content of Jonathan apples: *a*, Immediate storage at 32° F.; *b*, immediate storage at 36°; *c*, delayed 6 days at 65°, then stored at 32°; *d*, delayed 6 days at 65°, then stored at 36°; *e*, delayed 6 days at 65°, treated 2 days with 35 percent carbon dioxide, then stored at 32°. Chemical analyses were made in September 1934, at the beginning of the storage period; on November 5; and on January 14, 1935. Numbers on the curves represent percentages of soft scald appearing at the end of the storage period.

TABLE 1.—*Acetaldehyde content of pulp of Jonathan apples at various periods of storage in 1935–36*

Treatment	Acetaldehyde per 100 gm. of fresh pulp						Soft scald at end of storage
	September		October	November	December	January	
	Original sample	After prestorage treatment					
	Milligram	*Milligrams*	*Milligram*	*Milligram*	*Milligram*	*Milligram*	*Percent*
Immediate storage at 32° F.	0		0.0	0.0	0.0	0.0	9.5
Immediate storage at 36°	0		.0	.0	.0	.0	.0
Delayed 3 days at 70°; stored at 32°	0	0.0	.1	.0	.0	.0	3.6
Delayed 3 days at 70°; stored at 36°	0	.0	.1	.1	.0	.1	.0
Delayed 6 days at 70°; stored at 32°	0	.1	.0	.2	.0	.0	32.9
Delayed 6 days at 70°; stored at 36°	0	.1	.0	.1	.1	.0	.6
3 days at 70°; 2 days in 42–50 percent CO_2; stored at 32°	0	.8	.0	.1	.0	.0	.6
6 days at 70°; 2 days in 42–50 percent CO_2; stored at 32°	0	1.9	.0	.1	.1	.0	1.9

SUGAR

The results of the sugar analyses of juice for the 1933–34 and 1934–35 experiments are presented in table 2. In 1935–36 the apple pulp was analyzed. These results appear in table 3.

The prestorage treatment of the 1933 fruit produced no significant changes in the sugar constituents of the juice. By following the sugar content throughout the storage period, it will be seen that the trend of sucrose and total sugar is downward whereas that of reducing sugar is upward. The only lot that stands out from the others is the one that was delayed 11 days at 70° F. and then treated with carbon dioxide before final storage at 32°. This lot had the highest total sugar content throughout the storage period and developed no soft scald.

In the 1934 fruit (table 2) the reducing sugar fluctuated slightly during the storage period, but the trend appeared to be generally upward as in the experiment of the previous year. Total sugar values were somewhat higher at the end than at the beginning of the storage period. Sucrose values showed no consistent trend. Delayed storage appeared to increase the sucrose content of the juice. In these experiments the carbon dioxide treatment had no effect on the sugar content of the juice and there was no apparent relation between sugar changes and development of soft scald.

In the 1935 experiments (table 3) the prestorage treatment produced an immediate increase in sucrose and total sugar with no significant effect on reducing sugar except in lot 7. During the storage life of the fruit the reducing sugar, total sugar, and sucrose fluctuated irregularly. The drop in total sugar that occurred at the October sampling, except in lot 8, is difficult to explain. There was no significant fluctuation in moisture in any of the samples during the whole period. Only lots 1, 3, and 5 showed very high percentages of soft scald, and there was no apparent relation between changes in sugar content and the development of this disorder.

TABLE 2.—*Sugar content of juice of Jonathan apples at various periods of storage in 1933–35*

DATA FOR 1933-34

Treatment	Sugar per 100 ml. of juice												Soft scald at end of storage
	September						November			January			
	Original sample			After prestorage treatment									
	Reducing sugar	Sucrose	Total sugar	Reducing sugar	Sucrose	Total sugar	Reducing sugar	Sucrose	Total sugar	Reducing sugar	Sucrose	Total sugar	
	Grams	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Percent*
Immediate storage at 32° F	7.62	3.74	11.36				7.88	3.11	10.99	8.12	2.61	10.73	31.8
4 days at 70°, then 32°	7.62	3.74	11.36	7.69	3.96	11.65	7.50	3.42	10.92	8.23	2.28	10.51	47.3
4 days at 70°; 44 hours in 25–52 percent CO_2; then 32°	7.62	3.74	11.36	7.65	3.47	11.12	7.64	3.13	10.77	8.29	2.56	10.85	15.0
11 days at 70°, then 32° storage	7.62	3.74	11.36	7.90	3.64	11.54	7.62	3.34	10.96	8.13	2.40	10.53	18.3
11 days at 70°; 68 hours in 20–40 percent CO_2; then 32°	7.62	3.74	11.36	7.95	4.04	11.99	8.05	3.34	11.39	8.85	2.33	11.18	.0

DATA FOR 1934-35

Immediate storage 32°	7.95	3.08	11.03				8.40	3.38	11.78	8.50	2.75	11.25	0.2
Immediate storage 36°	7.95	3.08	11.03				7.93	3.46	11.39	8.74	3.07	11.81	.0
6 days 65°, then 32°	7.95	3.08	11.03	7.54	4.01	11.55	7.88	4.49	12.37	8.85	3.10	11.95	46.7
6 days 65°, then 36°	7.95	3.08	11.03	7.54	4.01	11.55	7.85	4.47	12.32	8.81	3.46	12.27	2.7
6 days 65°; 2 days in 35 percent CO_2; then 32°	7.95	3.08	11.03	7.84	3.75	11.59	8.26	3.61	11.87	8.60	2.81	11.41	14.2

TABLE 3.—*Reducing sugar, sucrose, and total sugar content of pulp of Jonathan apples after various periods of storage in 1935–36*

Lot No.	Treatment	September						October			November			December			January			Soft scald at end of storage
		Original sample			After prestorage treatment															
		Reducing sugar	Sucrose	Total sugar	Reducing sugar	Sucrose	Total sugar	Reducing sugar	Sucrose	Total sugar	Reducing sugar	Sucrose	Total sugar	Reducing sugar	Sucrose	Total sugar	Reducing sugar	Sucrose	Total sugar	
		Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
1	Immediate storage at 32° F	7.87	2.78	10.65	-------	-------	-------	6.41	2.89	9.30	8.44	3.81	12.25	8.19	3.32	11.51	8.37	3.39	11.76	9.5
2	Immediate storage at 36°	7.87	2.78	10.65	-------	-------	-------	6.57	3.20	9.77	8.34	3.75	12.09	8.51	3.80	12.31	6.84	2.81	9.65	.0
3	3 days at 75°, then 32° storage	7.87	2.78	10.65	7.90	4.10	12.00	6.67	3.23	9.90	8.50	3.89	12.39	8.55	3.24	11.79	6.84	2.66	9.50	3.6
4	3 days at 75°, then 36° storage	7.87	2.78	10.65	7.90	4.10	12.00	6.57	3.33	9.90	8.39	3.89	12.28	8.09	3.67	11.76	6.96	2.84	9.80	.0
5	6 days at 70°–75°, then 32°	7.87	2.78	10.65	7.84	4.34	12.18	7.45	4.00	11.45	8.19	3.80	11.99	8.47	3.38	11.85	8.26	2.52	10.78	32.9
6	6 days at 70°–75°, then 36°	7.87	2.78	10.65	7.84	4.34	12.18	7.14	3.75	10.89	8.03	3.67	11.70	8.07	3.12	11.19	8.39	2.52	10.91	.6
7	3 days at 70°; 2 days in 40 percent CO_2; then 32°	7.87	2.78	10.65	8.20	3.50	11.70	7.56	3.86	11.42	8.12	3.50	11.62	8.00	3.05	11.05	8.30	3.25	11.55	.6
8	6 days at 70°–75°; 2 days in 40 percent CO_2; then 32°	7.87	2.78	10.65	7.81	3.73	11.54	8.03	3.82	11.85	8.42	3.56	11.98	8.07	3.19	11.26	8.46	3.14	11.60	1.9

ACIDITY

In table 4 are shown the changes in total acid, pH value, and acid index during the storage period, as well as the total amount of soft scald and internal break-down occurring at the end of the storage period, in the 1933–34 experiments. As was to be expected, the total acid and hydrogen-ion concentration decreased as the period of storage progressed. The treatments involving delayed storage lowered the total acidity at once. In the November sampling the lots treated with carbon dioxide showed a lower acidity than the corresponding untreated lots, but in January this was true only of the lots that had been delayed 11 days prior to carbon dioxide treatment.

TABLE 4.—*Total acid, pH value, and acid index of juice of Jonathan apples stored under various conditions in 1933–34*

Treatment	September						November			January			Physiological disorders found at end of storage period	
	Original sample			After prestorage treatment										
	Total acid (as malic per 100 ml. of juice)	pH	Acid index ($\times 10^7$)	Total acid (as malic per 100 ml. of juice)	pH	Acid index ($\times 10^7$)	Total acid (as malic per 100 ml. of juice)	pH	Acid index ($\times 10^7$)	Total acid (as malic per 100 ml. of juice)	pH	Acid index ($\times 10^7$)	Soft scald	Internal break-down
	Gm.			*Gm.*			*Gm.*			*Gm.*			*Pct.*	*Pct.*
Stored immediately at 32° F.	0.759	3.34	40.3	-----	-----	-----	0.620	3.39	43.9	0.555	3.48	39.9	31.8	0.0
4 days at 70°, then 32°	.759	3.34	40.3	0.598	3.42	42.5	.600	3.41	43.1	.476	3.51	43.4	47.3	.0
4 days at 70°; 2 days in 25–52 percent CO_2; then 32°	.759	3.34	40.3	.609	3.42	41.7	.550	3.46	41.9	.496	3.61	33.0	15.0	1.9
11 days at 70°; then 32°	.759	3.34	40.3	.576	3.44	42.2	.520	3.49	41.2	.474	3.63	33.1	18.3	.0
11 days at 70°; 1 day at 98.6°-104°; then 32°	.759	3.34	40.3	.489	3.57	36.8	.500	3.56	36.8	.352	3.72	36.2	2.8	38.9
11 days at 70°; 3 days in 20–40 percent CO_2; then 32°	.759	3.34	40.3	.575	3.46	40.3	.470	3.51	43.8	.437	3.89	19.7	.0	11.7

The immediate effect of the heat treatment following the delay was to lower the total acidity and hydrogen-ion concentration. In September the heated lot showed less acidity than any other lot. This particular lot was next lowest in acidity at the November sampling and the lowest again in January. The heat treatment seemed to reduce the amount of soft scald but increased the percentage of internal break-down.

No relation between acidity of the apple juice and development of soft scald in the fruit was evident. This is true whether the amount of disease is compared with total acidity at any stage in sampling or with the rate of loss in acidity. The same is true of the hydrogen-ion values. The acid index was determined for each lot at the different sampling periods, but this figure offers no criterion for predicting the incidence of soft scald.

DISCUSSION

In the present investigation soft scald occurred under conditions usually considered conducive to the onset of this disorder; that is, a higher percentage of soft scald was found in the fruit stored immediately at 32° F. than in that stored at 36°, and holding at 65° to 70° prior to storage at both 32° and 36° greatly increased the percentage of soft scald unless the delay was followed by treatment with carbon dioxide. It would seem, then, that chemical analyses of the apples should indicate the manner in which these treatments have affected the physiology of the fruit. First it appears that, in general, delay disrupted the normal evolution of acetaldehyde. The carbon dioxide treatment following the delay always changed the rate of acetaldehyde evolution. The carbon dioxide treatments increased the acetaldehyde at the time of the first sampling following the treatment.

The relation between acetaldehyde content of apples and the incidence of physiological disorders is still not well understood. Although volatile substances such as acetaldehyde and alcohol have been shown to accumulate in small quantities during the normal storage life of apples, they may be increased tremendously by anaerobic conditions to the point where they become toxic to the apples; furthermore, fruits suffering from soft scald, soggy break-down, and internal breakdown have yielded, upon analysis, large quantities of acetaldehyde and alcohol. On the other hand, Thomas (*12*) states that ethyl alcohol and acetaldehyde accumulate in the unhealthy tissues of apples after the incidence of "low-temperature breakdown" and "soft or deep scald" but that these substances do not accumulate in apples or pears stored in air so long as the fruit remains physiologically healthy. In the present investigation no direct correlation was shown to exist between acetaldehyde content at any sampling and the ultimate development of soft scald.

No immediate effect of carbon dioxide on the acids in the juice was noted. Delay at high temperatures naturally reduced the acidity, but this result was not affected by the carbon dioxide treatment immediately following it. In the second sampling, however, the lots treated with carbon dioxide were lower in acid than those that had been delayed without subsequent carbon dioxide treatment. Plagge and Gerhardt (*10*) have reported that a small amount of soggy break-down is associated with a higher acid loss. It has been shown by several investigators that continued exposure of certain fruits to carbon dioxide lowers the acidity. The data presented here indicate that this effect upon acidity might be carried over for 2 months, yet they are rather meager to justify such a conclusion.

The sugar content of the juice or of the pulp of the apples at any of the sampling times showed no relation to the rate of soft-scald development. Plagge reported that one of the effects of delayed storage was to increase the sugar content of the juice. Hockey and Boyle (*7*) stated that the amount of sucrose and total sugars present in Gravensteins at the time of picking had little or no effect on the prevalence of "spot scald." In the present work the total sugar values at the time of the last sampling indicate a similarity between the delayed lots treated with carbon dioxide and those stored immediately at 32° F. It might be inferred that the changes in metabolism produced by the delayed storage were counteracted by the subsequent carbon

dioxide treatment. The results on soft scald suggest this also. A larger percentage of soft scald occurred in the delayed lots unless the delay was followed by treatment with carbon dioxide.

That the sugar, acid, acetaldehyde, and alcohol content of apples constitutes an index of the metabolic activity is suggested by the changes brought about by the various storage treatments, but no evidence has been found that these substances are determining factors in the development of soft scald.

SUMMARY

A physiological study was made of Jonathan apples stored under conditions known to influence the incidence of soft scald. These conditions consisted of immediate storage at 32° and 36° F., delays of 3 to 11 days at 65° to 75° prior to storage at the above-mentioned temperatures, and a carbon dioxide or a heat treatment following the delay but prior to storage at 32° and 36°.

Fruit stored immediately generally evolved acetaldehyde in small quantities but in slowly increasing amounts, whereas a delay at 65° to 75° F. greatly increased the amount of acetaldehyde during the early part of the storage life of the fruit. Treatment with carbon dioxide following the delay increased the acetaldehyde content of the fruit.

Delay at high temperatures (65° to 75° F.) tended to increase the sucrose and total sugar content of both juice and pulp samples at the beginning of the storage period. Treatment with carbon dioxide did not greatly influence reducing sugar and sucrose.

Delay reduced the acidity of the juice at the beginning of storage, and carbon dioxide treatment had no immediate additional effect. The lots treated with carbon dioxide showed a slightly lower acidity at the November sampling, which was approximately the middle of the storage period.

Heating the fruit to 98.6°–104° F. for 1 day following an 11-day delay at 70° lowered the total acidity and hydrogen-ion concentration. This lot of fruit showed less soft scald but more internal break-down than the other lots.

Although changes in sugar, acid, and acetaldehyde content of apples are doubtless an index of physiological activity, no correlation was found between these substances and the occurrence of soft scald.

LITERATURE CITED

(1) Association of Official Agricultural Chemists.
1930. official and tentative methods of analyses. Ed. 3, 593 pp., illus. Washington, D. C.

(2) Brooks, Charles, Cooley, J. S., and Fisher, D. F.
1926. diseases of apples in storage. U. S. Dept. Agr. Farmers Bul. 1160, 24 pp., illus.

(3) ——— and Harley, C. P.
1934. soft scald and soggy break-down of apples. Jour. Agr. Res. 49: 55–69, illus.

(4) Du Toit, M. S., and Reyneke, J.
1933. studies in the keeping quality of fruit, i. So. Africa Dept. Agr. Sci. Bull. 118, 44 pp.

(5) Harley, C. P., and Fisher, D. F.
1931. a study of the internal atmosphere of apples in relation to soft scald. Amer. Soc. Hort. Sci. Proc. (1930) 27: 271–275.

(6) HEALD, F. D., NELLER, J. R., OVERLEY, F. L., RUEHLE, G. D., and LUCE, W. A.
1928. ARSENICAL SPRAY RESIDUE AND ITS REMOVAL FROM APPLES AND PEARS. Wash. Agr. Expt. Sta. Bul. 226, 100 pp., illus.
(7) HOCKEY, J. F., and BOYLE, J. A.
1934. GRAVENSTEINS—TIME OF PICKING IN RELATION TO SPOT SCALD. Sci. Agr. 14: 608–613, illus.
(8) KIDD, FRANKLIN, and WEST, CYRIL.
1925. FUNCTIONAL DISEASES OF APPLES IN COLD STORAGE. [Gt. Brit.] Dept. Sci. and Indus. Res., Food Invest. Bd., Spec. Rpt. 23, 15 pp., illus.
(9) MILLER, ERSTON V.
1936. DISTRIBUTION OF ACETALDEHYDE AND ALCOHOL IN THE APPLE FRUIT. Jour. Agr. Res. 53: 49–55.
(10) PLAGGE, H. H., and GERHARDT, FISK.
1930. ACIDITY CHANGES ASSOCIATED WITH THE KEEPING QUALITY OF APPLES UNDER VARIOUS STORAGE CONDITIONS. Iowa Agr. Expt. Sta. Res. Bul. 131, pp. [342]–[358], illus.
(11) ——— MANEY, T. J., and PICKET, B. S.
1935. FUNCTIONAL DISEASES OF THE APPLE IN STORAGE. Iowa Agr. Expt. Sta. Bul. 329, pp. [35]–79, illus.
(12) THOMAS, MEIRION.
1931. THE PRODUCTION OF ETHYL ALCOHOL AND ACETALDEHYDE BY FRUITS IN RELATION TO THE INJURIES OCCURRING IN STORAGE. PART II. INJURIES TO APPLES AND PEARS OCCURRING IN THE PRESENCE OF OXYGEN AND IN THE ABSENCE OF ACCUMULATIONS OF CARBON DIOXIDE IN THE STORAGE ATMOSPHERE. Ann. Appl. Biol. 18: 60–74, illus.

A CYTOLOGICAL STUDY OF SOME SPECIES IN THE GENUS PASPALUM [1]

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INTRODUCTION

The genus *Paspalum* L. belongs to the large tribe Paniceae in the grass family, the Gramineae. With the exception of *Paspalum boscianum* Flügge, the 42 species occurring quite commonly in the southeastern part of the United States are perennials (*5*, *pp. 575–601*).[3] Many of these species make a valuable contribution to the native grass pasture in the Southeast, and *P. dilatatum* Poir., *P. notatum* Flügge, and *P. urvillei* Steud. are showing much promise in some of the improved pastures in this region.

A search of the literature reveals that only five species in this genus have been studied cytologically, and, with the exception of *Paspalum dilatatum*, those studied are of little importance in the Gulf States. The results of these studies, by Avdulov (*1*, *2*), Avdulov and Titova (*3*), Church (*4*), and Marchal (*8*), are presented in table 1.

The failure to find resistance to ergot (*Claviceps paspali* Stevens and Hall) in *Paspalum dilatatum* and several other promising species suggested the wisdom of resorting to species hybridization for the improvement of these grasses. It was with a desire to arrive at a better understanding of the cytology of the *Paspalum* species, and hence a clue as to which hybrid combinations might be expected to be most fertile, that this study was begun.

TABLE 1.—*Somatic chromosome numbers in Paspalum species as reported in the literature*

Species	Somatic chromosome number (2*n*)	Reference
P. dilatatum Poir	[1] 40	(*8*)
P. pubescens Muhl	[1] 20	(*4*)
P. scrobiculatum L	40	(*1*)
P. stoloniferum Bosc	[1] 20, 20–23	(*8*, *3*)
P. virgatum L	80	(*2*)

[1] Reported as the *n* number; the number here given is double that reported.

MATERIALS AND METHODS

All plant material used in this study was grown either in the grass introduction garden or on the premises of the Georgia Coastal Plain Experiment Station, Tifton, Ga. Since many obstacles were encoun-

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[3] Italic numbers in parentheses refer to Literature Cited, p. 197.

tered in the use of the acetocarmine technique, all studies reported here were made on root-tip material. Root tips collected between 8 and 10 a. m. were fixed in Craf fixative for 24 hours. The embedding procedure consisted of a slight modification of the alcohol-chloroform method as revised by La Cour (*6*). Sections were cut 10μ thick and

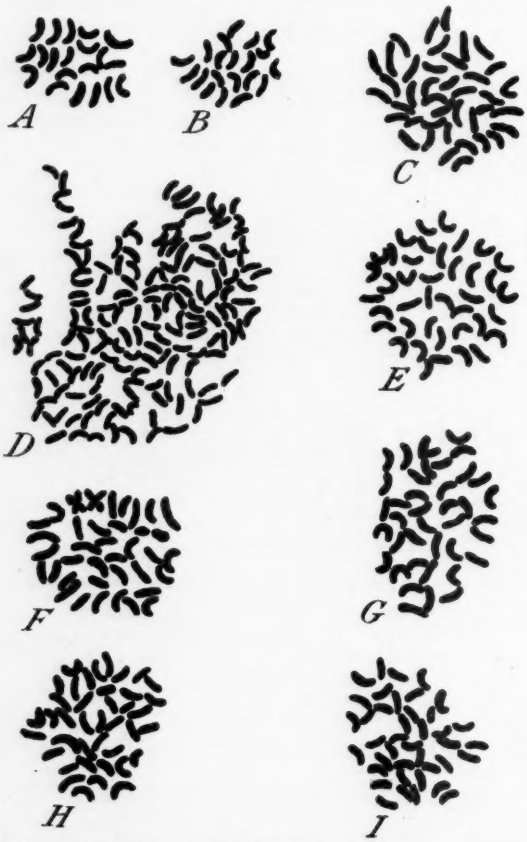

FIGURE 1.—Camera lucida drawings of chromosomes on equatorial plates in cells from root tips of nine species of *Paspalum: A, P. cilialifolium*, $2n=20$; *B, P. paniculatum*, P. E. I. 128190, $2n=20$; *C, P. dilatatum*, $2n=40$; *D, P. floridanum*, $2n=160$; *E, P. urvillei*, $2n=40$; *F, P. malacophyllum*, F. C. 04240, $2n=40$; *G, P. boscianum*, $2n=40$; *H, P. notatum*, $2n=40$; *I, P. notatum*, P. E. I. 121415, $2n=40$.

were stained by the iodine-gentian violet technique described by La Cour (*6*). With the exception of *Paspalum floridanum* Michx., all chromosome numbers were determined by making camera lucida drawings of three to five different equatorial plates in each species. Although much material of *P. floridanum* was examined, only in the equatorial plate shown in figures 1, *D*, and 2, *A*, were the chromosomes well enough separated to permit a count.

OBSERVATIONS

A list of the *Paspalum* species examined, together with their somatic chromosome numbers, is presented as table 2. The camera lucida drawings and photomicrographs from which chromosome numbers were determined are presented as figures 1 and 2. A brief description of each species follows.

TABLE 2.—*Somatic chromosome numbers in the Paspalum species examined at Tifton, Ga.*

Text figure No.	Species	Somatic chromosome number (2n)	Text figure No.	Species	Somatic chromosome number (2n)
1, *A*; 2, *B*	*P. ciliatifolium*	20	1, *F*	*P. malacophyllum*, F. C. 04240.	40
1, *B*	*P. paniculatum*, P. E. I. 128190.	20	1, *G*	*P. boscianum*	40
1, *C*	*P. dilatatum*	40	1, *H*	*P. notatum*	40
1, *D*; 2, *A*	*P. floridanum*	160	1, *I*	*P. notatum*, P. E. I. 121415	40
1, *E*	*P. urvillei*	40			

Paspalum ciliatifolium Michx.—This species, a native of the southeastern part of the United States, occurs in cleared or semicleared areas and supplies some of the best grazing to be found in many native pastures in Georgia and Florida. Since the data presented in tables 1 and 2 indicate that 10 is the basic chromosome number in this genus, the plants examined here may be considered diploid, $2n=20$ (figs. 1, *A*; 2, *B*).

Paspalum paniculatum, L., P. E. I.[4] 128190.—Seed of this species was sent to the United States Department of Agriculture from Navua, Fiji Islands, by Richard B. Howard in 1938. The grass has made a luxuriant growth in the greenhouse and is being tested in the introduction garden this year. This species proved to be diploid, $2n=20$ (fig. 1, *B*).

Paspalum dilatatum Poir.—Most widely used of all *Paspalum* species in improved pastures in the Gulf States, this grass was introduced into the Southern States from Uruguay or Argentina about the middle of the last century (*5*). The scarcity and cost of high-quality Dallis grass seed, a result of its susceptibility to ergot and its dependence upon a relatively high soil-nutrient level for satisfactory growth, are the principal factors limiting its use. The material examined was tetraploid, $2n=40$ (fig. 1, *C*). This agrees with the *n* number reported by Marchal (*8*).

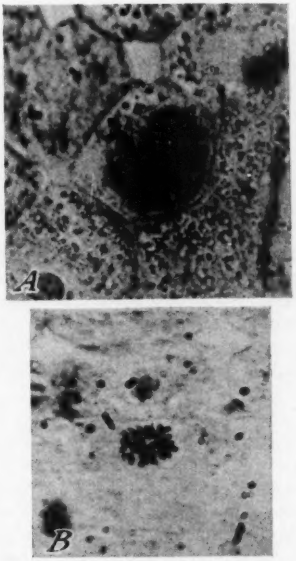

FIGURE 2.—Photomicrograph of equatorial plate in root tip of (*A*) *Paspalum floridanum* (× 700) and (*B*) *P. ciliatifolium* (× 850).

[4] Refers to accession number of the Division of Plant Exploration and Introduction.

Paspalum floridanum Michx.—This grass is one of the large-seeded, robust species of *Paspalum*, occurring in low, moist, sandy soil, flat woods, pine woods, and low prairies throughout most of the Coastal Plain south of Maryland (*5*). The huge ergots formed in the spikelets of this species demonstrate in a striking manner that this grass is not a source of genes for ergot resistance. Although only one figure could be found which was suitable for counting, the material examined, together with the apparent polyploid nature of the genus, leaves little doubt that this species is sixteenploid, $2n=160$ (figs.1, *D*; 2, *A*).

Paspalum urvillei Steud.—This erect bunch grass grows along ditches and roadsides and in moist and wet soils in most of the Southeastern States. Its heavy seed production, tolerance of poorly drained soils, and ability to make good growth on soils of low fertility are desirable features of this grass. Its failure to withstand close grazing, probably due to its upright growth habit, usually causes it to "go out" in improved pastures in this region. Cytologically it was found to be tetraploid, $2n=40$ (fig.1, E).

Paspalum malacophyllum Trin., F. C. 04240.—This species, introduced from Argentina a number of years ago, has been receiving considerable attention during the past few years. Although evidence is available that this erect bunch grass will go out when heavily grazed, its vigor, adaptability, and heavy seed production make it one of the most promising grass introductions under observation at Tifton. The failure of the writer to find ergot sclerotia on this grass when growing in association with heavily infected Dallis grass during the last 3 years at Tifton and the failure of Lefobvre (*7*) to obtain ergot infection upon inoculation, indicate that it is the best source of genes for ergot resistance now available. The material examined was found to be tetraploid, $2n=40$ (fig. 1, *F*).

Paspalum boscianum Flügge.—This rather succulent annual, found growing in moist or wet fields and along ditches and ponds in most of the Coastal Plain, proved to be tetraploid, $2n=40$ (fig. 1, *G*).

Paspalum notatum Flügge.—This species, introduced from the West Indies, Mexico, and parts of South America, is showing much promise as a permanent pasture grass on the sandy soils of Georgia and Florida. Productivity, extensive root system, strong feeding power, stoloniferous habit, and ability to withstand close grazing are outstanding features of this species. Plants which may be referred to as common Bahia grass were tetraploid, $2n=40$ (fig. 1, *H*).

Paspalum notatum Flügge, P. E. I. 121415.—Early in 1937, W. A. Archer sent to Washington, D. C., a packet of seed from Santissima, Trinidad, Paraguay, which was identified as *Paspalum notatum*. Not only were the seeds smaller than common Bahia grass, but the plants which grew from them differed so much from common Bahia that there was some question as to whether this might not be a different species. Its greater frost resistance and its ability to grow at lower temperatures than common Bahia grass were promising features of this introduction. Cytologically this grass was found to be tetraploid, $2n=40$ (fig. 1, *I*). The fertility of the hybrids with common Bahia grass produced in 1938 and of plants grown from seed in 1939 should help to explain the taxonomy of this grass.

RELATION BETWEEN AREA OF EQUATORIAL PLATE AND NUMBER OF CHROMOSOMES

The relationship between the area of the equatorial plate, calculated from average diameter measurements of the plates in figure 1, *A* to *I*, and the number of chromosomes in the plate is of interest. In the nine *Paspalum* species considered, the average calculated equatorial plate area for the diploid species was 180 mm.^{2}; for the tetraploid species, 415 mm.^{2}; and for *Paspalum floridanum*, the sixteenploid species, 1,451 mm.^{2} If the area of the equatorial plate were directly proportional to the chromosome number of the species, these areas would be 180, 360, and 1,440 mm.^{2}, respectively. Thus it is apparent that a positive correlation of rather high magnitude exists between equatorial plate area and chromosome number in the *Paspalum* species examined in this study.

SUMMARY AND CONCLUSIONS

With evidence that 10 is the basic (n) number of chromosomes the 12 *Paspalum* species considered fall into a polyploid series ranging from $2n$ to $16n$. In this group there are 4 diploids, 6 tetraploids, 1 octoploid, and 1 sixteenploid. *P. ciliatifolium*, *P. paniculatum*, *P. stoloniferum*, and *P. pubescens* are diploid; *P. dilatatum*, *P. notatum*, *P. malacophyllum*, *P. urvillei*, *P. boscianum*, and *P. scrobiculatum* are tetraploid; *P. virgatum* is octoploid; and *P. floridanum* is sixteenploid. The fact that all of the most promising pasture species are tetraploids is worthy of note. The rather close correlation that exists between the calculated areas of the equatorial plates in figure 1, *A* to *I*, and their chromosome numbers is interesting.

LITERATURE CITED

(1) Avdulov, N. P.
1928. [karyo-systematische untersuchung der familie gramineen]. All Union Cong. Bot., Moscow, 1928, Jour. pp. 65–67. [In Russian.]

(2) ———
1931. karyo-systematische untersuchung der familie gramineen. 428 pp., illus. Leningrad. (Trudy Prikl. Bot., Genet., i Selek. Prilozh. (Bul. Appl. Bot., Genet., and Plant Breeding Sup. 44)).

(3) ——— and Titova, N. N.
1933. additional chromosomes in paspalum stoloniferum bosco. Trudy Prikl. Bot., Genet., i Selek. (Bul. Appl. Bot. Genet., and Plant Breeding) (2) 2: [165]–172. [In Russian. English summary, pp. 171–172.]

(4) Church, George L.
1929. meiotic phenomena in certain gramineae. II. paniceae and andropogoneae. Bot. Gaz. 88: 63–84, illus.

(5) Hitchcock, A. S.
1935. manual of the grasses of the united states. U. S. Dept. Agr. Misc. Pub. 200, 1040 pp., illus.

(6) La Cour, Leonard
1937. improvements in plant cytological technique. Bot. Rev. 3: 241–258.

(7) Lefebvre, C. L.
1939. ergot of paspalum. Phytopathology 29: 365–367.

(8) Marchal, E.
1920. recherches sur les variations numériques des chromosomes dans la série végétale. 108 pp., illus. Bruxelles (Acad. Roy. de Belg. Cl. des Sci. Mém., 2d ser., t.4, fasc. 3.)

CHEMICAL EFFECT ON LEAD ARSENATE OF CERTAIN SALTS WHICH MAY BE PRESENT IN SOIL AND SPRAY WATERS[1]

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INTRODUCTION

The present practice by orchardists of applying several lead arsenate sprays during the growing season leads to a gradual deposition of appreciable quantities of arsenic on the trees. Most of the arsenic thus applied finds its way sooner or later to the soil where it may accumulate year after year, especially in soils where drainage is poor. Moreover, the recent tendency to apply arsenicals directly to the soil (*11*)[2] in comparatively large quantities in order to kill the larvae of Japanese beetle (*Popillia japonica* Newm.) and other grubs still further enhances the possibility of accumulating large quantities of arsenic in certain soils.

As long as the arsenic compound remains insoluble on the plant or in the soil, there is no danger of injury to fruit trees or crops. However, as soon as the lead arsenate comes in contact with water or water containing soluble salts, especially alkalies, chemical reactions may take place by which appreciable quantities of water-soluble arsenic may be released and become available for absorption by the plant roots. This so-called "soluble arsenic" may be in the form either of arsenic acid (H_3AsO_4) or water-soluble salts such as $K_2H\ AsO_4$, Na_2HAsO_4, $(NH_4)_2HAsO_4$, depending on the kinds of bases, capable of displacing lead from the $PbHAsO_4$ molecule, present in soil solution.

In addition to the salts present in soil, waters used for spraying insecticides, especially hard waters, in many instances contain appreciable quantities of salts such as sulfates, chlorides, carbonates, bicarbonates, and nitrates, which may react directly with lead arsenate, forming soluble arsenic.

It has been definitely established by Kilgore (*10*), Headden (*9*), Clinton and Britton (*4*), Smith (*16*), Swingle, Morris, and Burke (*17*), Ginsburg (*6, 7, 8*), and other investigators that arsenical injury to plants may be caused by soluble arsenic either originally present in the spray mixture or subsequently formed. That small quantities of soluble arsenic present in certain soils may prove injurious to fruit trees and other crops has been shown by Headden (*9*), Newton (*13*), Morris and Swingle (*12*), and others. Cooper and his associates (*5*) have found that the accumulation of arsenic in certain soils causes a marked reduction in the yield of certain crops. Reed and Sturgis (*14*) report difficulties encountered by farmers of southwest Louisiana in growing rice following cotton that has been dusted

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[2] Italic numbers in parentheses refer to Literature Cited, p. 205.

with calcium arsenate. Carter (*2*) reports hydrochloric acid, sodium silicate, and sodium hydroxide as the best solvents for the removal of lead arsenate residue from fruits.

Any chemical possessing the properties of decomposing lead arsenate in the spray mixture or in the soil is of immediate importance to the agriculturist, particularly the fruit grower. He is primarily interested to know the chemicals possessing such properties. The purpose of the investigation reported in this paper was, therefore, to determine which of the chemicals normally found in soils or spray waters react with lead arsenate to form soluble arsenic.

MATERIALS AND METHODS

Of the various compounds that one would reasonably expect to find in soils and spray waters, as indicated by Clarke (*3*), Russell (*15*), and Weir (*18*), some 50 outstanding salts were selected for testing. They consisted of several members of each of the following 10 groups: Phosphates, carbonates, bicarbonates, acetates, sulfates, nitrates, chlorides, silicates, sulfides, and sulfites. Chemically pure salts were used in all tests.

In order to determine the amount of soluble arsenic that each of these salts can form from lead arsenate, the following method was employed: A standard brand of acid lead arsenate was mixed at the rate of 2 gm. in 500 cc. of distilled water (approximately 3 pounds per 100 gallons) with approximate stoichiometrical proportions of each salt. The mixture was allowed to stand at room temperature for about 24 hours with frequent shaking. At the end of this period the mixtures were filtered and the soluble arsenic in the filtrate was determined as As_2O_3 by the standard iodine method as outlined in the official method (*1*). From the percent of As_2O_3 thus found the approximate percent of $PbHAsO_4$ decomposed was then calculated.

Approximate stoichiometrical proportions were employed in order to furnish a uniform basis for comparison. Since $PbHAsO_4$ has a higher molecular weight (347) than any one of the salts tested, the reacting concentrations of the salts were necessarily lower than that of lead arsenate, ranging from 0.084 percent for CaS to 0.258 percent for $BaSO_4$ as compared with 0.4 percent for $PbHAsO_4$. The stoichiometrical concentration of each salt varied according to its molecular weight as well as to its reactive chemical equivalent in regard to $PbHAsO_4$. The concentration of lead arsenate remained constant in all the tests.

In another series of tests the concentration of certain selected salts was increased to several times their stoichiometrical proportions, the lead arsenate remaining constant, and the soluble arsenic formed was again analyzed. The actual concentrations of the salts tested in the two series are given in the tables.

The procedure of preparing the samples for analysis was as follows: The required amount of each salt, based on its anhydrous content, was accurately weighed into a 1-liter flask and mixed with 500 cc. of distilled water. After a few minutes shaking the hydrogen-ion concentration of the solution was determined potentiometrically, by employing the hydrogen electrode. The lead arsenate was then added. The hydrogen-ion concentration was determined to ascertain whether or not the pH value of the solution, by itself, plays any part in decom-

posing lead arsenate. Duplicate samples were run for each analysis. Blanks consisting of the salt in the same concentration, but without lead arsenate, were also run. The averages of the analyses are presented in condensed form in the two tables.

RESULTS AND DISCUSSION

DECOMPOSITION OF LEAD ARSENATE

A comparison of the data in table 1, where the results from all the salts tested are presented, shows that the sulfates, nitrates, and acetates, a total of 19 salts, did not appreciably affect the decomposition of lead arsenate, as may be seen from the low percentages of As_2O_3 analyzed in the filtrates. This disclosure was rather surprising, as all the salts in these 3 groups, except $BaSO_4$, are water soluble. The next 5 groups of salts caused the following percentages of decomposition of lead arsenate: Chlorides about 5; silicates from 7.7 to 9.6; carbonates from 4.3 to 47; bicarbonates about 7; and sulfites from 1.4 to 17.8. The highest percent decomposition of $PbHAsO_4$ occurred in the presence of sulfides, ranging from 30 for CaS to 89.2 for Na_2S_5.

Most interesting results were obtained with the group of phosphates. The three phosphates of calcium caused no material increases in the decomposition of $PbHAsO_4$, as compared with the check where lead arsenate alone was used. Of the three phosphates of potassium, the tribasic (K_3PO_4) decomposed 35; the dibasic (K_2HPO_4) 17.8; and the monobasic (KH_2PO_4) only 1 percent of the lead arsenate. Almost identical results were secured with the corresponding phosphates of sodium (tests 47, 48, 49). In each of these two groups of alkali phosphates the percent of soluble arsenic formed was in direct proportion to the number of either sodium or potassium atoms and in inverse proportion to the number of hydrogen atoms present in the molecule.

EFFECT OF HYDROGEN-ION CONCENTRATION

Since the pH values of the salt solutions tested lie within a wide range, varying from 3.6 for $Ca(H_2PO_4)_2$ to 11.4 for Na_2S_5, the relationship was studied between the hydrogen-ion concentration of the salt and the percentage decomposition of $PbHAsO_4$. A close analysis of the results in table 1 shows that in the majority of cases salts with pH values of 8 or higher formed large amounts of soluble arsenic. On the other hand, salts with pH values of 7 or lower in most instances either did not affect the lead arsenate at all or only slightly increased its solubility. Thus of 19 salts ranging in pH values from 8 to 11.4, 17 decomposed from 7 to 89.2 percent of lead arsenate, while only 2, namely $Na_2S_2O_3$ and $CaSO_3$, with pH values of 8.7 and 9.3, respectively (tests 9, 36), did not increase considerably the decomposition of lead arsenate as compared with the check. Again, of 29 salts with pH values from 3.6 to 7.2, 23 decomposed only from 0.28 to 1.5 percent of $PbHAsO_4$. Here the 5 chlorides and 2 carbonates tested offer exceptions. Although the pH values of the chlorides ranged from 6.5 to 7.1, the percentages of lead arsenate decomposed were appreciable, varying from 4.73 to 4.98. The carbonates of Ca and Mg (pH 6.5 and 6.4) decomposed 4.3 and 8.26 percent of $PbHAsO_4$, respectively.

It should be noted, however, that these results were obtained with chemically pure salts in distilled water. Somewhat different reactions

might be obtained if the same salts were present in soils varying in pH values. Deviations from these results should also be expected in soils high in organic constituents, which may act as buffers in preventing changes in the hydrogen-ion concentration.

TABLE 1.—*Soluble arsenic formed from lead arsenate (2 gm. in 500 cc.) when mixed with different salts in approximate stoichiometrical proportions*

Test No.	Name of salt	Chemical formula of salt	Approximate[1] solubility of salt in water	Salt tested	pH of salt solution	As_2O_5 produced	$PbHAsO_4$ decomposed
			Percent	*Per cent*	*Per cent*	*Per cent*	*Percent*
1	Aluminum sulfate	$Al_2(SO_4)_3$	31.00	0.12	3.9	0.40	1.40
2	Ammonium sulfate	$(NH_4)_2SO_4$	71.00	.153	6.3	.13	.45
3	Barium sulfate	$BaSO_4$	Trace	.258	5.8	.15	.52
4	Calcium sulfate	$CaSO_4$	.18	.136	7.1	.14	.49
5	Copper sulfate	$CuSO_4$	20.00	.168	4.6	.17	.59
6	Magnesium sulfate	$MgSO_4$	26.00	.14	6.8	.11	.38
7	Potassium sulfate	K_2SO_4	8.50	.20	7.1	.15	.52
8	Sodium sulfate	Na_2SO_4	5.00	.165	6.9	.24	.84
9	Sodium thiosulfate	$Na_2S_2O_3$	74.00	.184	8.7	.30	1.05
10	Zinc sulfate	$ZnSO_4$	80.00	.196	6.5	.08	.28
11	Ammonium nitrate	NH_4NO_3	[2]	.184	5.7	.19	.66
12	Calcium nitrate	$Ca(NO_3)_2$	[2]	.188	5.7	.21	.73
13	Magnesium nitrate	$Mg(NO_3)_2$	[2]	.172	7.8	.24	.84
14	Potassium nitrate	KNO_3	13.00	.232	5.8	.35	1.22
15	Sodium nitrate	$NaNO_3$	73.00	.196	6.5	.39	1.37
16	Calcium acetate	$Ca(C_2H_3O_2)_2$	43.00	.168	6.7	.25	.87
17	Magnesium acetate	$Mg(C_2H_3O_2)_2$	[3]	.152	6.9	.50	1.72
18	Potassium acetate	$KC_2H_3O_2$	[3]	.208	6.3	.31	1.09
19	Sodium acetate	$NaC_2H_3O_2$	26.00	.176	6.9	.30	1.05
20	Ammonium chloride	NH_4Cl	29.00	.12	6.6	1.40	4.90
21	Calcium chloride	$CaCl_2$	59.00	.128	6.6	1.35	4.73
22	Magnesium chloride	$MgCl_2$	52.00	.108	6.5	1.72	4.98
23	Potassium chloride	KCl	28.00	.172	7.2	1.42	4.96
24	Sodium chloride	$NaCl$	35.00	.136	7.1	1.40	4.90
25	Magnesium silicate	$MgSiO_3$	[4]	.108	8.8	2.20	7.70
26	Potassium silicate	K_2SiO_3	[3]	.165	9.9	2.25	7.87
27	Sodium silicate	Na_2SiO_3	[3]	.128	10.2	2.75	9.63
28	Ammonium bicarbonate	NH_4HCO_3	12.00	.092	8.0	2.00	7.00
29	Potassium bicarbonate	$KHCO_3$	25.00	.116	8.2	2.15	7.55
30	Sodium bicarbonate	$NaHCO_3$	5.00	.096	8.1	2.11	7.38
31	Ammonium carbonate	$(NH_4)_2CO_3$	100.00	.11	8.9	5.80	20.30
32	Calcium carbonate	$CaCO_3$	.01	.116	6.5	1.20	4.30
33	Magnesium carbonate	$MgCO_3$	.01	.096	6.4	2.36	8.26
34	Potassium carbonate	K_2CO_3	99.00	.16	10.5	13.40	47.00
35	Sodium carbonate	Na_2CO_3	7.00	.12	10.7	13.40	47.00
36	Calcium sulfite	$CaSO_3$	.125	.14	9.3	.40	1.40
37	Sodium sulfite	Na_2SO_3	28.00	.144	10.0	5.10	17.80
38	Calcium sulfide	CaS	[5]	.084	10.1	8.60	30.00
39	Sodium sulfide, mono	Na_2S	15.00	.09	11.1	25.00	87.50
40	Sodium sulfide, penta	Na_2S_5	[3]	.24	11.4	25.00	89.20
41	Monocalcium phosphate	$Ca(H_2PO_4)_2$	[4]	.092	3.6	.31	1.10
42	Dicalcium phosphate	$CaHPO_4$	.02	.104	7.1	.57	2.00
43	Tricalcium phosphate	$Ca_3(PO_4)_2$	[5]	.12	6.9	.43	1.50
44	Monopotassium phosphate	KH_2PO_4	25.00	.104	5.5	.29	1.00
45	Dipotassium phosphate	K_2HPO_4	[3]	.132	8.5	5.10	17.80
46	Tripotassium phosphate	K_3PO_4	[3]	.162	9.5	10.00	35.00
47	Monosodium phosphate	NaH_2PO_4	[3]	.092	5.6	.31	1.10
48	Disodium phosphate	Na_2HPO_4	6.00	.112	8.5	5.40	18.90
49	Trisodium phosphate	Na_3PO_4	28.00	.128	9.8	9.00	31.50
50							
Check	Lead arsenate only				5.2	.18	.63

[1] When exact percentages are not known the following expressions are used: Soluble, slightly soluble, very soluble, decomposes.
[2] Very soluble.
[3] Soluble.
[4] Decomposes.
[5] Slightly soluble.

SOLUBILITY OF CHEMICALS

Of all the chemicals tested, only four, Ca_3PO_4, $BaSO_4$, $CaCO_3$, and $MgCO_3$, were not sufficiently soluble at room temperatures to dissolve completely in the volume of water used in the tests. The other com-

pounds were appreciably soluble, many of them very soluble, as indicated in table 1. If the degree of solubility alone is taken as a criterion for measuring decomposition of lead arsenate, there seems to be no direct relationship. Thus $MgCO_3$, 0.01-percent soluble, decomposed 8.26 percent, whereas $MgSO_4$, 26-percent soluble, decomposed only 0.38 percent of $PbHAsO_4$. Again, $NaNO_3$ and $NaC_2H_3O_2$, both very soluble, decomposed only 1.37 and 1.05 percent of lead arsenate, respectively. An outstanding example is offered by the phosphates. The K_3PO_4, of slight solubility, decomposed 35 percent, while KH_2PO_4, 25-percent soluble, decomposed only 1 percent of lead arsenate.

Apparently other factors, in addition to solubility, such as acidity or alkalinity, degree of ionic dissociation, position of the base of the salt in the "displacement series" with respect to lead, and solubility of the reaction products play their parts in determining the extent of reaction of the salt with lead arsenate.

INCREASED SALT CONCENTRATIONS

In seeking more information to explain adequately why some soluble salts, as well as several entire groups of salts, did not react with lead arsenate, it occurred to the writer that the small concentrations used might have been insufficient for the chemical reaction of these salts to take place. Accordingly, another series of tests was run with higher concentrations, using multiples of the stoichiometrical proportions of the salts, theoretically required to react with 2 gm. of acid lead arsenate. For these tests the potassium and magnesium salts of the three groups, nitrates, acetates, and sulfates, as well as the entire group of phosphates, were selected. The actual concentrations of the salts and the soluble arsenic formed are given in table 2.

TABLE 2.—*Soluble arsenic formed from lead arsenate (2 gm. in 500 cc.) when mixed with different salts in multiples of their approximate stoichiometrical proportions*

Test No.	Name of salt	Chemical formula of salt	Salt tested	Multiples of stoichiometrical proportions used	As_2O_3 formed	$PbHAsO_4$ decomposed
			Percent		*Percent*	*Percent*
51	Potassium sulfate	K_2SO_4	5.0	25.0	0.44	1.54
52	Magnesium sulfate	$MgSO_4$	5.0	35.7	.26	.91
53	Potassium nitrate	KNO_3	1.0	4.3	.36	1.26
54	Magnesium nitrate	$Mg(NO_3)_2$	1.0	5.8	.31	1.08
55	Potassium acetate	$KC_2H_3O_2$	5.0	24.0	.29	1.02
56	Magnesium acetate	$Mg(C_2H_3O_2)_2$	.4	2.6	.23	.81
57	Monocalcium phosphate	$Ca(H_2PO_4)_2$	.4	4.3	.86	3.01
58	Dicalcium phosphate	$CaHPO_4$	.4	3.8	.46	1.60
59	Tricalcium phosphate	$Ca_3(PO_4)_2$	.4	3.3	.45	1.57
60	Monopotassium phosphate	KH_2PO_4	.4	3.8	.25	.87
61	Monosodium phosphate	NaH_2PO_4	.4	4.4	.40	1.40
62	Dipotassium phosphate	K_2HPO_4	.4	3.0	7.30	25.55
63	Disodium phosphate	Na_2HPO_4	.4	3.6	9.60	33.60
64	Tripotassium phosphate	K_3PO_4	.4	2.4	16.50	57.75
65	Trisodium phosphate	Na_3PO_4	.4	3.1	15.10	52.85
66 Check	Lead arsenate only		.4	1.0	.22	.77

The results from the second series substantiate the first findings, namely, that nitrates, sulfates, acetates, and monobasic phosphates do not form soluble arsenic from $PbHAsO_4$, regardless of the concentrations employed. Thus, increasing the concentrations of K_2SO_4 and $KC_2H_3O_2$ to 5 percent, or about 24 times the amount previously used, did not appreciably change the low percentages of soluble arsenic found in the filtrates. Similar results were also shown by the phosphates. Increasing the quantities of NaH_2PO_4 and KH_2PO_4 to about 4 times the concentration used in the first series of tests, produced only 0.4 percent and 0.25 percent of As_2O_3, respectively. Of the 3 calcium phosphates, only $Ca(H_2PO_4)_2$ showed a small increase of soluble arsenic, giving 0.86 percent (test 57) of As_2O_3 as compared with 0.31 percent (test 41).

On the other hand, the dibasic and the tribasic phosphates of sodium and potassium, which formed large amounts of soluble arsenic in the first series of tests, still further increased the percentages of As_2O_3 in the filtrates of the second series, as may be seen by comparing tests 62, 63, 64, and 65 (table 2) with tests 45, 46, 48, and 49 (table 1).

SUMMARY AND CONCLUSIONS

Some 50 salts generally present either in soils or in spray waters were tested in the laboratory to determine to what extent they can form soluble arsenic when in contact with acid lead arsenate. The salts were mixed in various concentrations with constant quantities of lead arsenate (about 3 pounds per 100 gallons) and allowed to stand for 24 hours with frequent shaking. The filtrates of these mixtures were analyzed for soluble arsenic.

Of the 10 different groups of salts tested, the nitrates, sulfates, and acetates, without a single exception, proved relatively nonreactive with $PbHAsO_4$.

The chlorides, silicates, and bicarbonates produced moderate quantities of soluble arsenic.

Salts of carbonates and sulfides usually formed large percentages of soluble arsenic.

In the phosphate group, the three phosphates of calcium and the monobasic phosphates of sodium and potassium formed inappreciable quantities of soluble arsenic, whereas the dibasic and especially the tribasic phosphates of either sodium or potassium formed large amounts of soluble arsenic.

The percent of solubility of a salt in water is, by itself, not a determining factor in forming soluble arsenic. But, of a group of salts possessing the property of decomposing lead arsenate, the salt which is highly soluble in water will form more soluble arsenic than a salt which is only slightly soluble in water.

The hydrogen-ion concentration of the salt solution plays, with few exceptions, an important role in decomposing lead arsenate. Salts with pH values of 8 to 11.4 produced, with two exceptions, more soluble arsenic than did salts with lower pH values.

LITERATURE CITED

(1) ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS.
1935. OFFICIAL AND TENTATIVE METHODS OF ANALYSIS . . . Ed. 4, 710 pp., illus. Washington, D. C.

(2) CARTER, R. H.
193 . AN INVESTIGATION OF SOLVENTS FOR THE REMOVAL OF LEAD ARSENATE RESIDUES FROM FRUITS. Jour. Econ. Ent. 27: 848-853.

(3) CLARKE, FRANK WIGGLESWORTH.
1924. THE DATA OF GEOCHEMISTRY. U. S. Geol. Survey Bul. 770, Ed.5, 841 pp.

(4) CLINTON, G. P., and BRITTON, W. E.
1912. TESTS OF SUMMER SPRAYS ON APPLE AND PEACHES. Conn. State Agr. Expt. Sta. Ann. Rpt. (1911) 35: [347]-406, illus.

(5) COOPER, H. P., PADEN, W. R., HALL, E. E., ALBERT, W. B., ROGERS, W. B., and RILEY, J. A.
1931. EFFECT OF CALCIUM ARSENATE ON THE PRODUCTIVITY OF CERTAIN SOIL TYPES. S. C. Agr. Expt. Sta. Ann. Rpt. 44: 28–36, illus.

(6) GINSBURG, J. M.
1927. CHEMICAL STUDIES OF THE SULFUR-LIME DRY MIX SPRAY IN REGARD TO THE FORMATION OF WATER SOLUBLE ARSENIC. Jour. Econ. Ent. 20: 625–631.

(7) ———
1933. THE EFFECT OF DIFFERENT SOAPS ON LEAD ARSENATE IN SPRAY MIXTURES. Jour. Agr. Res. 46: 179–182.

(8) ———
1937. EFFECT OF DIFFERENT SOAPS ON FORMATION OF SOLUBLE ARSENIC FROM LEAD ARSENATE IN SOFT AND HARD WATER. Jour. Econ. Ent. 30: 583–590.

(9) HEADDEN, WM. P.
1908. ARSENICAL POISONING OF FRUIT TREES. Colo. Agr. Expt. Sta. Bul. 131, 27 pp., illus.

(10) KILGORE, B. W.
1891. ON THE CAUSE AND PREVENTION OF THE INJURY TO FOLIAGE BY ARSENITES TOGETHER WITH A NEW AND CHEAPER ARSENITE, AND EXPERIMENTS ON COMBINING ARSENITES WITH SOME FUNGICIDES. N. C. Agr. Expt. Sta. Bul. 77b (Tech. Bul. 2), 11 pp.

(11) LEACH, B. R.
1926. EXPERIMENTS WITH CERTAIN ARSENATES AS SOIL INSECTICIDES. Jour. Agr. Res. 33: 1–8.

(12) MORRIS, H. E., and SWINGLE, D. B.
1927. INJURY TO GROWING CROPS CAUSED BY THE APPLICATION OF ARSENICAL COMPOUNDS TO THE SOIL. Jour. Agr. Res. 34: 59-78, illus.

(13) NEWTON, J. H.
1926. THE EFFECT OF SODIUM ARSENITE UPON THE GROWTH AND PROTECTION OF CERTAIN CROP PLANTS. Colo. Agr. Expt. Sta. 17th Ann. Rpt. of the State Entomologist for 1925, pp. 69–79.

(14) REED, J. FIELDING, and STURGIS, M. B.
1936. TOXICITY FROM ARSENIC COMPOUNDS TO RICE ON FLOODED SOILS. Amer. Soc. Agron. Jour. 28: 432–436.

(15) RUSSELL, SIR E. JOHN.
1932. SOIL CONDITIONS AND PLANT GROWTH. Ed. 6, 636 pp., illus. London, New York [etc.]

(16) SMITH, C. M.
1923. EXCRETIONS FROM LEAVES AS A FACTOR IN ARSENICAL INJURY TO PLANTS. Jour. Agr. Res. 26: 191–194.

(17) SWINGLE, D. B., MORRIS, H. E., and BURKE, EDMUND.
1923. INJURY TO FOLIAGE BY ARSENICAL SPRAY MIXTURES. Jour. Agr. Res. 24: 501–538, illus.

(18) WEIR, WILBERT WALTER.
1936. SOIL SCIENCE: ITS PRINCIPLES AND PRACTICE, INCLUDING BASIC PROCESSES FOR MANAGING SOILS AND IMPROVING THEIR FERTILITY. 615 pp., illus. Chicago and Philadelphia.

FURTHER STUDIES ON GROWTH SUBSTANCES IN RELATION TO THE MECHANISM OF THE ACTION OF RADIATION ON PLANTS[1]

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INTRODUCTION

It has been repeatedly demonstrated that ultraviolet and visible violet radiation have definite formative effects upon growing plants. One of the most striking of these effects is the general stunting of the plant. This was especially evident in the work of Schanz (*12*),[2] Popp (*7*), and Popp and Brown (*8, 9, 10*). In all this work no effect has been more consistently evident, except destruction by the extremely short wave lengths, than the decreased stature of the plants exposed to the blue-violet end of the spectrum.

In a previous study, Popp and McIlvaine (*11*), in an attempt to explain this formative effect of the short wave lengths, exposed seedlings to radiations from a mercury-vapor lamp in quartz, using the unscreened arc and three types of Corning glass filters: Noviol O, transmitting down to 389 mμ; G586A, transmitting the region 300–436 mμ; and red-purple Corex A, transmitting the region 250–415 mμ, These plants were tested for growth substance by a modified Went technique (*3, 14*), as were control plants kept in the dark. It was found that the wave length of radiation, the stature of the plants, and the amount of growth substance revealed by the tests, were all positively correlated. The shorter the wave length, the shorter were the plants and the smaller the amount of growth substance found. It seemed evident that if the Went technique is a reliable quantitative test for growth substance, then radiations, especially of the shorter wave lengths, have a destructive influence on the growth substance in the plant. This explains at least part of the mechanism of the action of radiation on plants. The present investigation was undertaken in continuation of the above study. In the work herein described more attention was given to the visible region of the spectrum. A study was also made of the effect on growth-substance content when seedlings were transferred from light to darkness and from darkness to light.

As early as 1933 there was presented some evidence indicating that light had some effect on growth substance. All this work, however, dealt with more mature plants. It was reported (*1, 5, 6, 13, 15*) that there is a higher concentration of growth substance in young illuminated plants than in those kept in the dark. Skoog and Thimann (*13*) concluded that light favors the formation of the growth substance in plants, and darkness favors its physiological activity. Later Avery, Burkholder, and Creighton (*2*), using fully matured

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[2] Italic numbers in parentheses refer to Literature Cited, p. 215.

plants, found that the growth substance disappeared in darkness, but reappeared when the plant was replaced in the light. They concluded that light is necessary for the synthesis of the growth substance. They also reported some evidence that red light especially favors such synthesis.

Burkholder and Johnston (*4*) reported in a paper reviewed more fully elsewhere (*11*) that growth substance was destroyed by the shorter wave lengths of light, after such substance had been allowed to diffuse into agar blocks. They also found that such light inhibited the growth of seedlings, and by making correlated tests for the growth substance, produced some evidence that this inhibition was the result of the inactivation of the growth substance.

EXPERIMENTAL PROCEDURE

The plants used for the tests were turnips (*Brassica rapa* L.), of the Purple Top White Globe variety. Selected seeds were germinated on moist cotton and filter paper in sterile Petri dishes, 50 seeds to a dish. The seedlings were kept in darkness at 25° C. except during the radiation treatments. In some cases other conditions were maintained. These are noted below.

The sources of radiation were (1) daylight, (2) a 500-watt Mazda lamp in reflector, (3) a General Electric mercury-vapor lamp in glass, rated at 100 watts, and (4) a mercury-vapor lamp in quartz. The intensity of the last-mentioned lamp at 50 cm. with no filter was 21.65 (10^{-5}/cm.^{2}) watts. In table 1 are shown the types of Corning glass filters used, with their spectral ranges of transmission in the visible and ultraviolet regions and the comparative amount of energy they transmit, the transmission of the violet being unity. The total energy transmitted by each of these glasses was measured by means of a pyrheliometer of the Kimball and Hobbs type [3] and either the distance from the lamp or the time of exposure, or both, were altered to equalize the energy through the filters. In some cases cheesecloth screens were used to lower intensities. The time and distance of exposure were based upon exposure to the unscreened mercury-vapor arc, this latter being enough to provide a maximum of the formative effects in the plant with a minimum of injury.

TABLE 1.—*Spectral range (visible and ultraviolet regions) and energy transmission* [1] *of the filters used*

Filter	Spectral range	Comparative energy transmitted from—	
		Mercury-arc source	Mazda source
	mμ		
G34	529–720	5	20
G403ED	374–585	5	8
Corex D, clear	250–720	22	30
H R Signal Red	610–685	13	24
Sextant Green	465–600	4	5
Violet	380–482	1	1

[1] Violet as unity.

[3] Lent by the Eppley Laboratory, Inc.

The plants were usually exposed to the particular radiation 24 hours after being placed in the germinators and semidaily or daily thereafter for 3½ or 7 days. During the radiation a strong current of air was kept moving over the plants to minimize the heating effects of the lamp and to disperse the ozone when the latter was formed by the mercury-vapor lamp in quartz. Controls consisted of plants grown under the same conditions maintained for the test plants, except that irradiations were omitted.

Two groups of plants were kept in diffused daylight in the greenhouse with various periods of darkening, to determine the effect of darkness following growth in the light. In this case controls consisted both of plants grown in total darkness and plants grown continuously under greenhouse conditions.

Tests for the growth substance were made by a modified Went *Avena* curvature method (*3*, *14*). The test coleoptiles were from oats (*Avena sativa* L.) of a uniform pure line, received from Dr. C. F. Noll, of the Pennsylvania Agricultural Experiment Station. In the growth-substance determinations about 1 mm. of the tip of each test seedling was cut off. These tips were then placed unilaterally on the ends of *Avena* coleoptiles which had been decapitated and placed in a dark moist chamber 2 hours previously. The coleoptiles bearing the test tips were placed in a moist chamber in the dark. Two hours later they were removed and the curvature of the coleoptile, if any, was measured in a shadow box. During all the manipulations the *Avena* seedlings were secured in special glass holders. All manipulations were carried out in photographically inactive light. That this light had no formative effect upon the *Avena* coleoptiles was determined by previous tests.

As a further check, growth substance was allowed to diffuse into agar blocks from the tips of turnip seedlings grown in the dark. These blocks were then placed in a moist chamber and irradiated by a mercury-vapor lamp in quartz through a screen of clear Corex D (2 mm.) glass. After this they were placed unilaterally on prepared, decapitated *Avena* coleoptiles which were then treated in the usual manner, described above. The control consisted of the same procedure, omitting only the irradiation of the blocks.

RESULTS

In table 2 are given the results of tests on seedlings grown for 7 days, receiving the radiations of a 500-watt Mazda lamp at 82 cm. for a total of 720 minutes every 24 hours. To minimize the heating effect, this radiation was administered 15 minutes at a time, alternating with 15-minute periods of darkness. Records of the mean heights of the seedlings were kept, and it will be noted that these were positively correlated with the amounts of growth substance indicated by the tests. These two filters, G34 and G403ED, divide the visible spectrum into two regions, there being only a small overlap in their regions of transmission. G34 transmits the red half of the spectrum and G403ED, the blue-violet half. Seedlings exposed to the shorter wave-lengths were uniformly shorter and contained less growth substance than those under the G34 glass.

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TABLE 2.—*Mean curvatures obtained from turnip test plants 3½ and 7 days of age under various conditions of radiation when screens dividing the visible spectrum into 2 parts were used*

Screen	Tests	Mean height of seedlings	Mean curvature
	Number	*Centimeters*	*Degrees*
PLANTS AGED 7 DAYS, AFTER RECEIVING RADIATIONS FROM A 500-WATT MAZDA LAMP A TOTAL OF 720 MINUTES EVERY 24 HOURS			
G34	768	5.63	4.43±0.34
G403ED Blue	768	5.01	3.68± .31
PLANTS AGED 3½ DAYS, AFTER RECEIVING RADIATIONS FROM A MERCURY-VAPOR LAMP IN QUARTZ FOR 5 MINUTES EVERY 12 HOURS			
G34	720	3.59	4.09±0.02
G403ED Blue	720	2.60	3.00± .21
PLANTS AGED 3½ DAYS, AFTER RECEIVING RADIATIONS FROM A 500-WATT MAZDA LAMP FOR A TOTAL OF 480 MINUTES EVERY 12 HOURS AND RADIATIONS FROM A MERCURY-VAPOR LAMP IN GLASS A TOTAL OF 60 MINUTES EVERY 24 HOURS			
G34	732	3.75	4.28±0.30
G403ED Blue	780	2.51	3.09± .21
Control (total darkness)	840	6.20	5.81± .26

In table 2 are also shown the results of irradiating the seedlings through the same screens but with a mercury-vapor lamp in quartz as the source of radiation. Exposures were for 5 minutes every 12 hours, and plants were grown for 3½ days. In this case there was a greater difference between the two series of experiments than when the source was a Mazda lamp. The shorter plants were those under the blue glass, and these also contained less of the growth substance.

In table 2 are shown the results obtained from plants grown under radiation from both types of sources. The seedlings received the radiations from the Mazda lamp for a total of 480 minutes every 12 hours for 3½ days. In addition, they received the radiation from a mercury-vapor lamp in glass at 15 cm. for a total of 60 minutes every 24 hours during the same period. The difference between the two groups was greater than in either of the preceding cases. The seedlings under the blue glass were here, also, shorter and contained less growth substance.

Several series of tests were made in which the visible spectrum was divided into three parts by the use of red (H R Signal Red), green (Sextant Green), and violet screens and comparisons made with the full spectrum (Corex D). In these series the total radiation reaching the plants was equalized on the basis of the total energy transmitted through the filters. This did not take into account differences of transmission in the infrared region, and since the total transmission of the Sextant Green screen and of the Violet screen were extremely low as compared with the H R Signal Red screen, results obtained with these screens are only partly to be attributed to the wavelength region in the visible spectrum transmitted by each of these screens. In fact, from previous results reported, it seems likely that even when the total intensities were equalized under the three screens the plants under the green and the violet screens received less visible radiation than did those under the red screen.

TABLE 3.—*Mean curvatures obtained from turnip test plants 7 days of age under various conditions of radiation when screens dividing the visible spectrum into 3 parts were used*

PLANTS THAT RECEIVED RADIATION FROM A 500-WATT MAZDA LAMP FOR A TOTAL OF 720 MINUTES EVERY 24 HOURS

Screen	Tests	Mean height of seedlings	Mean curvature
	Number	*Centimeters*	*Degrees*
Corex D (2 mm.)	576	1.60	1.27±0.027
H R Signal Red	576	2.97	3.48± .39
Sextant Green	576	4.89	5.64± .32
Violet	576	4.55	5.00± .17

PLANTS THAT RECEIVED RADIATION FROM A 500 WATT MAZDA LAMP FOR A TOTAL OF 720 MINUTES EVERY 24 HOURS AND FROM A MERCURY-VAPOR LAMP IN QUARTZ A TOTAL OF 15 MINUTES EVERY 24 HOURS

H R Signal Red	480	3.02	3.21±0.25
Sextant Green	480	4.95	5.43± .29
Violet	480	1.96	2.12± .22
Control (total darkness)	792	8.30	5.90± .17

PLANTS EXPOSED TO DAYLIGHT THROUGH PLATE GLASS AND WATER AND OTHER SCREENS

Corex D (2 mm.)	288	1.49	1.92±0.27
H R Signal Red	288	3.50	3.92± .37
Sextant Green	288	5.21	5.56± .33
Violet	288	5.00	4.96± .28

The results obtained with these screens are presented in table 3, which gives first the results obtained when a Mazda lamp was used as the source of radiation. The plants were irradiated for a total of 720 minutes every 24 hours. The radiation was in periods of 15 minutes alternating with 15-minute periods of darkness. The shortest plants were those under the clear Corex D screen, and these plants also contained the smallest amount of growth substance. Next in height and amount of growth were the seedlings under the H R Signal Red filter. The plants under the Violet screen were still taller and contained more growth substance, while those under the Sextant Green glass were the tallest and contained the most growth substance.

It is interesting to note that the plants under the Sextant Green screen as well as those under the Violet screen were taller and gave higher curvatures in the *Avena* test than did those under the red screen in spite of the fact that those under the red screen received only longer wavelengths of radiation. This may be attributed to the factors mentioned in a previous paragraph as well as to the fact that the Mazda lamp is relatively deficient in blue-violet radiation. That this is true is indicated in table 3, where the radiation from the Mazda lamp was supplemented by that from a mercury-vapor lamp which is rich in blue-violet rays. In this case, the plants under the violet screen were the shortest and contained the smallest amount of growth substance. Plants under the red glass were next in both respects, and those under the Sextant Green screen were tallest and contained the most growth substance. Controls in complete darkness exceeded the test plants in every case. The higher intensity of the blue-violet region under these conditions did not offset the effects under the Sextant Green screen

since this screen does not transmit much of the extreme blue-violet region.

Table 3 contains the results of growing plants in sunlight for one-half day and in diffused daylight one-half day every 24 hours for 7 days, through August. Four different screens were used over separate plant cultures, but, in addition, over all the plants was a plate-glass screen covered with a flowing film of cold water to minimize the heating effects of the sunlight. Under these conditions seedlings under the clear Corex D screen were the shortest and contained the smallest amount of growth substance. Next in height and amount of growth substance were those under the red glass. Seedlings under the violet screen came next in height and amount of growth substance, and the tallest plants, with the greatest amount of growth substance, were those under the green filter. Since intensities were not accurately equalized in this group, the greater height of the plants under the green and violet screens may be attributed to the low amount of radiation transmitted by them.

Table 4 contains the results of irradiating agar blocks into which had diffused growth substance from the tips of turnip seedlings grown in total darkness, the exposure being one 15-minute period at 50 cm. The amount of active growth substance in such blocks was not appreciable. The control blocks produced the usual amount of active growth substance. The *Avena* test coleoptiles were used at the age of 3½ days, during all of which time they had been growing in the dark.

Two series of experiments were carried out to determine the effect of various periods of light and darkness upon seedlings. In the case of the seedlings itemized in table 5, all tests were made at the end of 7 days. There was some evidence of accumulation of growth substance, but the amount was evidently small. The smallest amount was found in seedlings grown in diffused daylight for 7 days. The control had the largest amount and the other two groups were between these. Those in the light the last 3 days had less growth substance than those in the dark the last 3 days.

TABLE 4.—*Mean curvatures obtained from Avena coleoptiles 3½ days of age after irradiating the test blocks* [1] *with a mercury-vapor lamp in quartz*

Item	Tests	Mean curvature
	Number	*Degrees*
Irradiated agar blocks	480	0.02±0.00
Control	492	5.84±0.17

[1] Containing diffused growth substance from tips of turnip seedlings grown in total darkness.

TABLE 5.—*Mean curvatures obtained from turnip test plants 7 days of age after exposure to various conditions of diffused daylight and darkness*

Conditions	Tests	Mean height of seedlings	Mean curvature
	Number	*Centimeters*	*Degrees*
Control (total darkness)	588	5.40	5.08±0.42
In dark 4 days, then in light 3 days	600	3.75	3.32± .35
In light 4 days, then in dark 3 days	576	4.58	4.80± .32
In diffused daylight 7 days	588	3.00	2.39± .33

In table 6 are shown the results of various periods of diffused daylight and darkness in three series of experiments. These seedlings were grown in soil in the greenhouse, and the days are reckoned from the time the plants appeared above the soil. Each series can be considered as a single culture. For instance, in series A, about 700 seeds were planted. These had been above the ground for 3 days when 132 of them were tested for the growth substance. The rest were placed in darkness for 3 days, at the end of which time another sample (132) of them was tested. The remaining seedlings were placed in diffused daylight and at the end of 3 days more these were tested. Controls were grown for the 9 days in either complete darkness or in diffused daylight, with no changes. There was little accumulation of the growth substance evident from the results obtained. Seedlings that had been in the dark always contained more of the growth substance than did those that had been in the light, regardless of conditions existing previously.

TABLE 6.—*Mean curvature obtained from turnip seedlings transferred from light to darkness and from darkness to light*

Series and conditions	Tests	Mean curvature
Series A:	*Number*	*Degrees*
After 3 days of diffused daylight	132	2.46±0.20
After 3 days of darkness following the 3 days in daylight	132	4.90±.14
After 3 more days of diffused daylight following the 3 days in darkness	132	2.61±.17
Control—9 days of diffused daylight	144	2.55±.23
Control—9 days of darkness	132	5.03±.27
Series B:		
After 2 days of diffused daylight	144	2.53±.18
After 4 days of darkness following the 2 days in daylight	144	4.96±.17
After 2 more days of diffused daylight following the 4 days in darkness	144	2.89±.21
Control—8 days of diffused daylight	144	2.48±.18
Control—8 days of darkness	144	5.11±.26
Series C:		
After 4 days of diffused daylight	132	2.40±.19
After 4 days of darkness following the 4 days in daylight	132	4.79±.16
After 3 more days of diffused daylight following the 4 days in darkness	132	2.72±.21
Control—11 days of diffused daylight	132	2.45±.16
Control—11 days of darkness	132	4.99±.24

DISCUSSION

Throughout the experiments there was found to be a very positive correlation between the heights of the seedlings and the amounts of growth substance present in them as indicated by the *Avena* curvature technique. It was also found that under equal amounts of energy the heights of the plants were less when the seedlings were exposed to shorter wave lengths of radiation. Similarly, the shorter the wave lengths of radiation, the smaller was the amount of growth substance present. When the visible spectrum was divided into three sections by the use of red, green, and violet screens, the plants under the green screen were the tallest and contained the most growth substance. This probably may be attributed to the fact that the radiation passing through these screens was not equalized in the visible region of the spectrum but on the basis of total energy transmitted, including infrared. Furthermore, the relative deficiency of blue-violet radiation in the Mazda lamp source caused plants under the violet screen to be taller than those under the red screen (table 3). The greater height of the plants under the green screen and the greater amount of growth

substance they contained might be thought to indicate that green light has less inactivating effect on growth substance than does red light, but this cannot be proved until plants have been grown under both kinds of light under accurately equalized energy.

When the visible spectrum was roughly divided into two parts, by the use of the G34 and G403ED screens, and intensities equalized, the seedlings under the blue glass were always shorter and contained less of the growth substance than did those under the yellow glass. The source of radiation caused some of the differences between the two. It is evident from the data that the blue end of the spectrum has an inhibiting effect on elongation and a correlated inactivating effect on growth substance. When the source was a Mazda lamp, the difference between the two groups was significant, although not great. When sources richer in the blue and violet radiations were used, the amount of growth substance and the height of the seedlings were considerably less than in the plants under the G34 screen. It is believed safe to assume that the lesser height of the plants under the blue screen is caused by a greater inactivation of the growth substance by the shorter wave lengths of radiation. It is also believed that the red light has some inactivating effect, although not nearly so much as blue-violet light.

In the tests in which seedlings were transferred from light to darkness and from darkness to light, the plants always contained less growth substance after being in the light and more after being in the dark. Although several periods of light and darkness were used, results were always the same. Obviously in seedlings of this age, light causes inactivation of the growth substance and darkness favors its production or activity.

SUMMARY

This study was carried out in continuation of an earlier one by Popp and McIlvaine. The previous work had indicated that the short wave-length radiation, from 250–415 mμ, inhibited the growth of seedlings by inactivating the growth substance in the shoot tips. The present work enlarges the study by showing the effect of radiation of wave lengths 374–720 mμ.

A number of turnip seedlings were grown and subjected to visible radiation and to various conditions of daylight and darkness. Heights of the plants were recorded, and the amount of growth substance present after the treatments was determined by a modified Went *Avena* curvature technique. The effect of the shorter wave-length radiation on growth substance in agar was similarly determined.

It was found that when the visible spectrum was divided in half, under approximately equalized energy, plants grown under the blue-violet half were shorter and contained less growth substance than those under the red half.

When the spectrum was divided into three parts (red, green, and blue violet), it was not possible under the conditions used in these experiments to accurately equalize energy in the visible region. Consequently, the plants under the green region were the tallest, probably having received the least light. Whenever a source rich in blue-violet rays was used, the plants under the blue violet were always the shortest In all cases, the tallest plants had the most growth substance and the shortest the least.

Growth substance allowed to diffuse from turnip-stem tips into agar was inactivated by the radiation from a mercury-vapor lamp.

When seedlings were transferred from light to darkness and from darkness to light, they always contained less growth substance after being in the light and more after being in the dark.

In general, these results substantiate the conclusions of the authors in their previous paper that the formative effect of radiation may be at least partly explained on the basis of the effect of radiation on growth substances in the plant.

LITERATURE CITED

(1) AVERY, GEORGE S., JR.
1935. DIFFERENTIAL DISTRIBUTION OF A PHYTOHORMONE IN THE DEVELOPING LEAF OF NICOTIANA, AND ITS RELATION TO POLARIZED GROWTH. Torrey Bot. Club. Bul. 62: 313–330, illus.

(2) ——— BURKHOLDER, PAUL R., and CREIGHTON, HARRIET B.
1937. GROWTH HORMONE IN TERMINAL SHOOTS OF NICOTIANA IN RELATION TO LIGHT. Amer. Jour. Bot. 24: 666–673.

(3) BOYSEN-JENSEN, P.
1936. GROWTH HORMONES IN PLANTS . . . Transl. and rev. by George S. Avery, Jr., and Paul R. Burkholder . . . 268 pp., illus. New York and London.

(4) BURKHOLDER, PAUL R., and JOHNSTON, EARL S.
1937. INACTIVATION OF PLANT GROWTH SUBSTANCE BY LIGHT. Smithsn. Misc. Collect. 95, No. 20, 14 pp., illus.

(5) NAVEZ, A. E.
1933. GROWTH PROMOTING SUBSTANCES AND ILLUMINATION. Natl. Acad. Sci. Proc. 19: 636–638.

(6) OVERBEEK, J. VAN.
1933. WUCHSSTOFF, LICHTWACHSTUMSREAKTION UND PHOTOTROPISMUS BEI RAPHANUS. Rec. des Trav. Bot. Néerland. 30: [537]–626, illus.

(7) POPP, HENRY WILLIAM.
1926. A PHYSIOLOGICAL STUDY OF THE EFFECT OF LIGHT OF VARIOUS RANGES OF WAVE LENGTH ON THE GROWTH OF PLANTS. Amer. Jour. Bot. 13: 706–736, illus.

(8) ——— and BROWN, F.
1936. EFFECTS OF DIFFERENT REGIONS OF THE VISIBLE SPECTRUM UPON SEED PLANTS. *In* Duggar, B. M., ed., Biological Effects of Radiation . . . , v. 2, ch. 22, pp. 763–790. New York and London.

(9) ——— and BROWN, F.
1936. THE EFFECT OF ULTRA-VIOLET RADIATION UPON SEED PLANTS. *In* Duggar, B. M., ed., Biological Effects of Radiation . . . , v. 2, ch. 26, pp. 853–887. New York and London.

(10) ——— and CHARLTON, FLORENCE BROWN.
1938. EFFECTS OF ULTRAVIOLET RADIATION UPON GERMINATION AND SEEDLING DEVELOPMENT. Pa. Agr. Expt. Sta. Bul. 366, 50 pp. illus.

(11) ——— and MCILVAINE, H. R. C.
1937. GROWTH SUBSTANCES IN RELATION TO THE MECHANISM OF THE ACTION OF RADIATION ON PLANTS. Jour. Agr. Res. 55: 931–936.

(12) SCHANZ, FRITZ.
1919. WIRKUNGEN DES LICHTS VERSCHIEDENER WELLENLÄNGE AUF DIE PFLANZEN. Deut. Bot. Gesell. Ber. 37: 430–442, illus.

(13) SKOOG, F., and THIMANN, K. V.
1934. FURTHER EXPERIMENTS ON THE INHIBITION OF THE DEVELOPMENT OF LATERAL BUDS BY GROWTH HORMONES. Natl. Acad. Sci. Proc. 20: 480–485.

(14) WENT, F. W.
1928. WUCHSSTOFF UND WACHSTUM. Rec. des Trav. Bot. Néerland. 25: 1–116, illus.

(15) ———
1935. AUXIN, THE PLANT GROWTH-HORMONE. Bot. Rev. 1: 162–182, illus.

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